NHR-8 regulated P-glycoproteins uncouple xenobiotic stress resistance from longevity in chemosensory *C. elegans* mutants

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Abstract

Longevity is often associated with stress resistance, but whether they are causally linked is incompletely understood. Here we investigate chemosensory defective *Caenorhabditis elegans* mutants that are long-lived and stress resistant. We find that mutants in the intraflagellar transport protein gene *osm-3* were significantly protected from tunicamycin-induced ER stress. While *osm-3* lifespan extension is dependent on the key longevity factor DAF-16/FOXO, tunicamycin resistance was not. *osm-3* mutants are protected from bacterial pathogens, which is *pmk-1* p38 MAP kinase dependent while TM resistance was *pmk-1* independent. Inhibition of P-glycoproteins with verapamil suppressed tunicamycin resistance and expression of P-glycoprotein xenobiotic detoxification genes was elevated in *osm-3* mutants. The nuclear hormone receptor *nhr-8* was necessary and sufficient to regulate P-glycoproteins and tunicamycin resistance in a cholesterol-dependent fashion. We thus identify a cell-nonautonomous regulation of xenobiotic detoxification and show that separate pathways are engaged to mediate longevity, pathogen resistance, and xenobiotic detoxification in *osm-3* mutants.
Introduction

Chemosensation is a genetically tractable phenotype in various model organisms. In C. elegans, many mutants with defective chemosensation have been identified. Sensory phenotypes are complex in nature and many of the classical chemosensory mutants were originally characterized by their behavioral phenotypes. Odr mutants for example have an abnormal odorant response while Osm (osmotic avoidance abnormal) mutants do not avoid high salt environments (Bargmann, 2006). In mutants like tax-4 and odr-1, mutations in components of neuronal G-protein coupled receptor (GPCR) signaling cause atypical chemosensory behavior (Bargmann, 2006). These mutants are not only characterized by a failure to adequately respond to their environment, but show additional phenotypes linked to various life traits including pathogen resistance, increased lifespan and drug detoxification (Gaglia et al., 2012, Apfeld and Kenyon, 1999, Dent et al., 2000). Many chemosensory mutants are long-lived and this phenotype depends on the DAF-16/FOXO transcription factor that is regulated by the insulin signaling pathway (Apfeld and Kenyon, 1999).

Beyond mutations in GPCRs, variants in genes involved in the development of amphid sensory neurons can lead to chemosensory defects. One example are mutations in intraflagellar transport (IFT) proteins that prevent full cilia development of amphid sensory neurons (Inglis et al., 2007). Alterations in the IFT genes osm-3 and daf-10 physically disrupt the development of neuronal dendrites that project toward the tip of the nose where they are exposed to the outer environment (Inglis et al., 2007). Thus, IFT mutants are usually characterized by defects in the chemical perception of their environment. Further, developmental defects in lumen formation of the amphid head channel in daf-6 mutants prevent the direct contact of amphid sensory neurons with the outside (Perens and Shaham, 2005).
In wild type (WT) worms, amphid and phasmid sensory neurons can be visualized using the red lipophilic dye DiI, which fluorescently stains them bright red. The dye is passively taken up by sensory neurons when they are fully developed and in contact with the environment. Dye filling defective (Dyf) *C. elegans* are defined by an inability to take up DiI into their sensory neurons (Inglis et al., 2007). The Dyf phenotype arises in *daf-6* animals and also in many IFT mutants. Interestingly, Dyf mutants have unique stress resistance phenotypes that act via different signaling pathways. One such Dyf mutant, *daf-10(m79)*, has a unique resistance to pathogenic bacteria that was proposed to be downstream of sensory input (Gaglia et al., 2012). Further, pairs of amphid head neurons, such as ASH and ASJ that often become disrupted in Dyf mutants help coordinate the innate immune response to bacterial stress after infection (Meisel et al., 2014). Longevity is often associated with activation of physiological stress pathways and has been shown to be regulated by the insulin signaling pathway in Dyf *C. elegans* (Apfeld and Kenyon, 1999).

Conserved mechanisms of longevity and stress resistance have additionally been linked to hyposmia in higher model organisms. In *Drosophila melanogaster*, loss of the putative chemoreceptor Or83b has been shown to significantly increase lifespan (Libert et al., 2007). Further, mice with ablated olfactory sensory neurons, have a unique metabolic signature that protects them from high-fat diets (Riera et al., 2017). While the olfactory machinery is seemingly more complex in higher organisms, *C. elegans* have unique phenotypes associated with the loss or alteration of their sensory neurons. First, unbiased screens for drug resistance have independently identified the Dyf phenotype as a common feature in drug resistant *C. elegans* (Fujii et al., 2004, Menez et al., 2016, Collins et al., 2008). Selection for resistance to the anthelmintic drug ivermectin was shown to enrich for Dyf mutants in several forward genetic screens (Menez et al., 2016, Page, 2018, Dent et al., 2000). Follow-up work
identified P-glycoproteins, a class of ATP binding cassette (ABC) transporters, as the drivers behind ivermectin resistance in Dyf mutants (Ardelli and Prichard, 2013).

Second, some chemosensory mutants, such as daf-10, show resistance to pathogenic bacteria (Gaglia et al., 2012).

Here, we characterize a long-lived Dyf mutant, osm-3, that was previously identified in a forward genetic screen for resistance to the potent ER stressor tunicamycin (TM).

From this screen, we reported the role of the hexosamine biosynthetic pathway in TM resistance and longevity (Denzel et al., 2014). Our aim was to uncover the mechanism by which Dyf mutants are resistant to TM. We found that the TM resistance of osm-3 mutants is independent from its longevity and pathogen resistance phenotypes.

Further, we showed that P-glycoproteins mediate TM resistance in Dyf animals and we found that the nuclear hormone receptor nhr-8 mediates osm-3 drug resistance.

The “Green Theory” of ageing proposes that the accumulation of toxic endo- and xenobiotic debris contributes to physiological decline with age (Afschar et al., 2016, Gems and McElwee, 2005). Our work highlights drug resistance as a unique characteristic of Dyf mutants and functionally uncouples it from known stress response pathways that have been implicated in stress resistance and longevity of chemosensory mutants.
Results

Dye filling defective *C. elegans* mutants are resistant to TM

TM is commonly used to induce endoplasmic reticulum (ER) stress by inhibiting the addition of N-glycans to nascent polypeptides (Parodi, 2000, Heifetz et al., 1979). In *C. elegans*, where the ER machinery is conserved, treatment with TM is toxic and induces the ER unfolded protein response. In fact, on plates containing TM at concentrations above 4 μg/mL, newly hatched larvae die at early developmental stages (Shen et al., 2001). Previously, we had carried out an ethyl methane-sulfonate (EMS) mutagenesis screen to identify TM-resistant and longlived *C. elegans* mutants (Denzel et al., 2014). The largest cohort of classifiable mutants presented dye filling defects (Dyf) (Figure 1A). Dyf mutants are uniquely characterized by their inability to take up the lipophilic red fluorescent dye DiI in their ciliated amphid neurons (Inglis et al., 2007).

Among the Dyf mutants from the TM resistance screen, we found new alleles of genes previously linked to ciliary development. The mutant allele we selected for our investigation, *osm-3(dh441)IV* (this allele will be referred to as *osm-3*), carries a premature stop in the eighth exon, which prevents normal cilia development causing the Dyf phenotype (Figure 1A, Figure 1-figure supplement 1A). *osm-3* is a member of the kinesin family involved in axonal transport and development and mutants are known to show a Dyf phenotype (Inglis et al., 2007). While WT worms fail to develop on TM at concentrations above 4 μg/mL, *osm-3* mutants fully develop at concentrations at least up to 10 μg/mL TM (Figure 1B). Loss of OSM-3, as well as other Dyf mutations, promote longevity in *C. elegans* (Apfeld and Kenyon, 1999). Demographic lifespan analysis of *osm-3* indeed confirmed extended lifespan in our point mutant (Figure 1C and Supplementary Table 1). *osm-3* mutants, and Dyf mutants
in general, have never been described as TM resistant especially at concentrations as high as 10 μg/mL.

To further explore whether the Dyf phenotype is linked to TM resistance we tested other Dyf mutants that were previously described as long-lived in the literature (Apfeld and Kenyon, 1999). To our surprise, all Dyf mutants that we tested proved to be significantly TM resistant at the WT lethal dose of 10 μg/mL TM (Figure 1D,F). In contrast, the long-lived chemosensory mutants odr-1(n1936)X and odr-3(n2150)V that display no Dyf phenotype were only slightly TM resistant (Figure 1E,F, Figure 1-figure supplement 1B-J). Our data thus suggest phenotypic differences regarding TM resistance among the larger class of chemosensory defective mutants.

ER stress response is blunted in osm-3 mutants

TM treatment activates the ER unfolded protein response (UPRER); we thus characterized the overall transcriptional response of osm-3 mutants upon TM treatment. We performed transcriptomic analyses on osm-3 and WT worms after 6 hours of TM treatment. Notably, the gene ontology (GO) terms related to ER stress and ER protein folding showed significant upregulation in WT animals but not in osm-3 mutants (Figure 2A). qPCR analysis of UPR target genes further confirmed that there was no UPRER induction in the TM-treated osm-3 mutants compared to the WT (Figure 2-figure supplement 1). A hallmark of UPRER induction in C. elegans is the upregulation of the molecular chaperone HSP-4/BiP (Shen et al., 2001). Upon TM treatment of osm-3 mutants carrying an hsp-4::GFP reporter construct, there was no significant increase in the GFP signal compared to the untreated control, while in the WT the GFP levels were significantly increased (Figure 2B,C). This observation corroborates the results from the transcriptome analysis.
One potential explanation for a suppressed UPRER response in osm-3 mutants might be a general defect in the stress signaling pathway. To address this possibility, we sought to activate the UPR by alternate means. The ER membrane protein SEL1/HRD3 is a cofactor of the HRD1 ubiquitin ligase complex involved in ER-associated degradation (ERAD) (Hampton et al., 1996). Using sel-1 RNAi to inhibit ERAD, we observed a robust activation of the hsp-4::GFP reporter, suggesting a functional UPRER response upon ER stress (Figure 2D,E). We further used DTT treatment to induce ER stress and found normal levels of hsp-4::GFP induction and xbp-1 splicing (Figure 2F) in osm-3 mutants. Together, these observations rule out that the reduced response to TM observed in osm-3 mutants was due to a defect in UPR signaling.

**TM resistance in osm-3 mutants is independent from daf-16 or the PMK-1/p38 MAPK pathway**

The lifespan extension observed in chemosensory defective C. elegans as well as in Drosophila has been shown to be at least partially insulin signaling dependent (Apfeld and Kenyon, 1999, Libert et al., 2007). Therefore, we performed a demographic lifespan analysis to determine the role of the insulin signaling pathway in the lifespan extension of osm-3 mutants. Indeed, the osm-3 lifespan extension was fully daf-16 dependent, as the lifespan of the osm-3; daf-16 double mutants was identical to the daf-16 lifespan (Figure 3A, Supplementary Table 1). To our surprise, the osm-3; daf-16 double mutant was resistant to TM while daf-16 single mutants do not develop at 10 µg/mL TM (Figure 3B). A previous study by Henis-Korenblit et al. had shown elevated hsp-4::GFP levels during daf-16 knockdown in a daf-2 mutant (Henis-Korenblit et al., 2010). We therefore asked whether the knockdown of daf-16 would sensitize osm-3 mutants to TM. Interestingly, knock-down of daf-16 by RNAi did not rescue the hsp-4::GFP response after TM treatment (Figure 3-figure supplement 1).
Given the links between the insulin signaling pathway and the ER stress signaling (Henis-Korenblit et al., 2010, Kyriakakis et al., 2017, Matai et al., 2019, Labbadia and Morimoto, 2014), we were further surprised to find that daf-2 mutants failed to develop on 10 µg/mL TM while osm-3 mutants fully developed (Figure 3C). Together, this evidence suggests that the osm-3 lifespan extension and TM drug resistance are uncoupled and instead appear to act via independent or parallel pathways.

In C. elegans, Pseudomonas aeruginosa PA14 is a pathogen that is often used to study innate immunity. Of note, UPRER targets have been implicated in the innate immune response of C. elegans during PA14 infection (Richardson et al., 2010, Haskins et al., 2008). As the ER plays a role in innate immunity and as TM disrupts ER function, we speculated that osm-3 mutants would be more robust on PA14 than WT. Indeed, osm-3 mutants were more resistant to PA14 than WT, however this increased pathogen resistance was suppressed by loss of the PMK-1 p38 mitogen-activated protein kinase (MAPK) pathway (Figure 3D). With this in mind, we tested whether pmk-1 mutation could suppress the osm-3 TM resistance. The osm-3; pmk-1 double mutant was fully resistant to TM, while the pmk-1 single was sensitive (Figure 3E). Taken together, insulin signaling and the MAPK pathway, two major stress response pathways in C. elegans that have links to ER protein quality control, are not required for TM resistance in osm-3 mutants.

P glycoprotein inhibition suppresses osm-3 TM resistance

Given the lack of ER stress in TM-treated osm-3 mutants along with the genetic separation of TM resistance from insulin or MAPK signaling, we hypothesized that TM might be cleared from worm tissues through xenobiotic detoxification. To test this, we used verapamil (VPL) to inhibit P-glycoproteins (PGPs) that secrete toxins. PGPs are a conserved family of ATP binding cassette (ABC) transporters found on the cell
membrane (Sangster, 1994) and *C. elegans* have 15 PGP genes. VPL has been used to specifically inhibit PGP activity, re-sensitizing worms to the antihelmintic compound ivermectin (Menez et al., 2016). Indeed, 1 nM VPL significantly suppressed development of *osm-3* *C. elegans* in the presence of TM, while having no effect on controls without TM (Figure 4A).

Dyf *C. elegans* mutants have been independently identified in drug resistance screens (Fujii et al., 2004, Menez et al., 2016, Dent et al., 2000). Consistent with these findings, we observed that *osm-3* mutants were resistant to 200 nM methyliogen dichloride (paraquat), as they fully develop at a concentration that is toxic to WT *C. elegans* (Figure 4B). Similarly, *osm-3* mutants fully developed to adults in the presence of 6 µg/mL ivermectin that is lethal to WT animals (Figure 4C). In contrast, *osm-3* mutants showed no difference to WT animals in heat stress assays or in the presence of hydrogen peroxide (H_2O_2) suggesting that *osm-3* mutants are not resistant to non-xenobiotic stressors (Figure 4-figure supplement 1A,B). While paraquat and hydrogen peroxide are both oxidative stressors, paraquat acts indirectly via the mitochondria (Castello et al., 2007) while hydrogen peroxide is a primary cause for oxidative damage. Thus, we view paraquat as a xenobiotic stressor. Given the involvement of PGP-dependent xenobiotic detoxification in TM resistance of *osm-3* mutants, we wondered about its role in the longevity of *osm-3* mutants. Notably, VPL did not shorten *osm-3* lifespan (Figure 4D). Moreover, VPL did not affect WT lifespan (Figure 4D and Supplementary Table 1) and had no visible effect on WT development (Figure 4-figure supplement 1C). Together, these data support the conclusion that the xenobiotic stress resistance and longevity phenotypes of *osm-3* mutants are mediated by independent pathways.

**NHR-8 signaling regulates xenobiotic detoxification response through PGPs**
VPL treatment suggested a role for PGPs in the TM resistance of osm-3 mutants. Using quantitative PCR we found a significant upregulation of pgp-3, pgp-5, pgp-8, pgp-11, and pgp-13 in osm-3 mutants (Figure 5A). The nuclear hormone receptor NHR-8 has been linked to xenobiotic detoxification and PGP regulation in C. elegans (Menez et al., 2019, Lindblom et al., 2001). Indeed, we found that the upregulation of PGP expression in osm-3 mutants was suppressed in the osm-3; nhr-8 double mutant (Figure 5A). This led us to ask whether osm-3 TM resistance was dependent on NHR-8 signaling. Indeed, the osm-3; nhr-8(hd117)IV double mutant was not resistant to TM while the osm-3 mutant fully developed (Figure 5B), suggesting that NHR-8 is necessary for osm-3 TM resistance. We next asked if NHR-8 was sufficient for TM resistance using a transgenic overexpressor (Magner et al., 2013). Of note, we found a significant upregulation of pgp-8 in nhr-8 transgenic animals (Figure 5C). In a developmental TM dose response assay, we found that overexpression of nhr-8 in WT worms results in significant TM resistance (Figure 5D). Characterization of UPRER signaling using the hsp-4::GFP reporter showed that pgp-8 RNAi sensitizes osm-3 mutants to TM. This experiment was done at 20 µg/mL TM to induce a mild ER stress in osm-3 mutants. Together, these data suggest that TM stress resistance in osm-3 mutants is due to effective xenobiotic detoxification that is mediated by NHR-8 and PGPs. Consistent with previous studies (Magner et al., 2013), we found that nhr-8 mutation shortened lifespan and that it, likewise, shortened osm-3 survival (Figure 5-figure supplement 1).

Sterol signals modulate TM resistance

We next wanted to further understand the systemic signals that control xenobiotic detoxification of TM. The NHR-8 ligand remains unknown, but is likely a sterol molecule derived from cholesterol metabolism (Magner et al., 2013). We thus tested the role of
cholesterol in TM resistance and found that omitting cholesterol from the culture plate for one generation leads to TM hypersensitivity in both WT and osm-3 animals (Figure 6A). Developmental cholesterol dose response assays in the presence of TM confirmed that WT TM resistance is cholesterol dependent (Figure 6B). In nhr-8 and osm-3; nhr-8 double mutants, however, cholesterol treatment failed to protect from TM developmental toxicity. This demonstrates a key role of NHR-8 in mediating TM resistance downstream from sterol signals. To corroborate a role of cholesterol derived signals in TM resistance, we used the hsp-4::GFP reporter and found that high cholesterol significantly reduced TM-induced ER stress (Figure 6C). Consistent with this, pgp-8 was upregulated by high cholesterol treatment (Figure 6D). These observations suggest cell-nonautonomous regulation of xenobiotic detoxification of TM by cholesterol-derived ligands that enhance PGP expression via NHR-8.
Discussion

In this study, we found that osm-3 mutants as well as other Dyf mutants are resistant to the ER toxin TM. Instead of an activated stress signaling status that might explain the TM resistance, osm-3 Dyf mutants show no UPR\textsuperscript{ER} induction upon TM treatment. Consistent with previous findings (Apfeld and Kenyon, 1999) the lifespan extension of osm-3 was daf-16 dependent. Despite the established link between the insulin signaling pathway and stress resistance, the TM resistance in osm-3 mutants was not insulin signaling dependent. Further, while osm-3 mutants were pathogen resistant, this resistance was fully dependent on the PMK-1/p38 MAPK pathway. We were surprised that neither of the two pathways weakened the TM resistance phenotype. Dyf C. elegans drug resistance is not specific to TM, as we also observed resistance to paraquat and ivermectin. While paraquat and ivermectin resistance have been previously reported in Dyf mutants (Dent et al., 2000, Fujii et al., 2004, Menez et al., 2016), no studies have demonstrated similar resistance mechanisms in Dyf C. elegans mutants to TM. Our findings show that this resistance is due to increased PGP expression in Dyf worms. Importantly, we demonstrate regulation of PGPs by cholesterol and the nuclear hormone receptor NHR-8. Broad inhibition of PGPs using verapamil or loss of nhr-8 significantly re-sensitized osm-3 mutants to TM. Consistently, NHR-8 overexpression or high cholesterol treatment resulted in TM resistance in WT animals.

Given that improved protein homeostasis is one of the cellular hallmarks of longevity (Lopez-Otin et al., 2013), we presumed that selection for TM resistance would serve as a proxy phenotype for longevity given that TM specifically targets ER protein folding. More specifically, we expected that high fidelity ER protein quality control would be the driver of longevity in these mutants. Previous findings describing increased hexosamine biosynthetic pathway flux (Denzel et al., 2014) or constitutive XBP-1
activation (Taylor and Dillin, 2013) have linked protein homeostasis to longevity and stress resistance (Denzel and Antebi, 2015); therefore the connection between TM resistance and longevity seemed apparent. Given that Dyf mutants are resistant to pathogenic bacteria through enhanced ER homeostasis, we also speculated that TM resistance might serve as a proxy phenotype for pathogen resistance. On the contrary, we found that xenobiotic detoxification via PGP s is sufficient for TM resistance but not longevity in osm-3 mutants. pmk-1 and daf-16 were required for pathogen resistance and longevity, respectively. Whether the longevity phenotype of osm-3 mutants would be suppressed by nhr-8 disruption is difficult to assess as nhr-8 mutants themselves show decreased survival (Magner et al., 2013).

Adding another dimension to the cell-nonautonomous regulation of innate immunity (Aballay, 2013), we propose that the nervous system regulates systemic xenobiotic detoxification C. elegans, likely through a sterol ligand. Our data suggest that mutants with defective amphid neurons have increased PGP expression. Several PGPs have been shown to be expressed in non-neuronal tissue (Lincke et al., 1993, Sheps et al., 2004). Moreover, we found that PGP expression is regulated by nhr-8, which has also been shown to be expressed in the intestine of C. elegans (Magner et al., 2013). Combining this previous information with our finding that Dyf mutants are resistant to several uniquely toxic drugs, we conclude that neuronal signaling controls drug resistance through nuclear hormone signaling. Given our observation that supplementation with cholesterol is itself sufficient for TM resistance in WT worms one might further investigate the link between neuronal states and the specific cholesterol-derived signal that drives these drug resistance phenotypes.

PGPs are likely regulated in C. elegans to help them combat toxins found in their natural habitat. Toxic metabolites are undoubtedly common in the soil where C. elegans are found. The drugs used in our study, ivermectin (a derivative of
avermectin) (Burg et al., 1979) and TM, were first discovered as antibiotics that are produced by soil bacteria (Takatsuki et al., 1971). Because *C. elegans* have no adaptive immune response, having PGPs as part of their innate immune system allows them to clear toxic molecules they may encounter in the wild. While Dyf mutants do not occur in the wild, our data nonetheless demonstrate a link environmental sensing and drug resistance.

In humans, PGPs have been implicated in drug resistant malignancies (Lehne, 2000). Using *C. elegans* as a tool to study the cross talk between tissues, one might be able to better understand how extracellular signaling drives ABC transporter expression in chemotherapy resistant cancer. Further studies in cell culture might also further characterize TM, as well as other toxic metabolites, as substrates for *C. elegans* PGPs.
### Key Resources Table

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Worm maintenance

_C. elegans_ nematodes were cultured using standard husbandry methods at 20°C on nematode growth media (NGM) agar plates seeded with _E. coli_ strain OP50 unless otherwise stated (Brenner, 1974).

Dye filling assay in _C. elegans_

The dye filling assay was performed on a synchronized population of day 1 adults. 40-60 adult worms were placed in M9 containing 10 μg/mL DiI. They were left at room temperature for two hours in the staining medium, then transferred to NGM plates seeded with _E. coli_ OP50. The worms were then allowed to crawl on the food for one hour to allow for the dye to be cleared from the gut. The worms were scored for dye filling using a Leica SPX-8 confocal microscope or a Leica fluorescent stereo-microscope. Images were taken using the confocal microscope.

Drug resistance assays

NGM plates containing either TM, paraquat or ivermectin were used in developmental assays to test for resistance. To this end, we performed a 4h egg lay on NGM plates at room temperature then transferred the eggs to control and drug containing plates and recorded the number of eggs per plate. After incubation for four days at 20°C, plates were scored by counting the total number of L4 larvae or adults on each plate.
Paraquat plates were prepared by adding 1M methyl viologen dichloride (paraquat) directly onto seeded NGM plates for a final concentration of 200 μM and allowed to dry immediately before use. Ivermectin plates were prepared by adding ivermectin directly to the NGM medium at a final concentration of 6 μg/mL before pouring the plates. Modified NGM plates containing no cholesterol were made by using standard NGM without the addition of cholesterol. NGM agar plates containing 5, 25 or 50 μg/mL Cholesterol were used for development when specifically stated. These modified NGM plates were then supplemented with drugs as described above. All of the drug development assays were performed using *E. coli* OP50 or *E. coli* HT115 bacteria. For the developmental TM dose response assay with the *nhr-8* transgenic dhEx451 strain in Figure 5D, the transgene transmission rate was reported on a separate plate for each condition that was then used for the calculation of the successfully developed proportion of animals.

**ER stress quantification**

Synchronized day 1 adults were transferred to control and TM containing NGM plates seeded with OP50. After 6 hours of stress induction hsp-4::GFP levels were measured by large particle flow cytometry in both the WT and *osm-3*(dh441)*IV* background using the Union Biometrica Biosorter and imaged using a Leica fluorescent stereo-microscope. For RNAi experiments, animals were raised on control and *daf-16, sel-1* or *pgp-8* RNAi. Synchronized day 1 adults were transferred to vehicle or TM containing plates seeded with control or experimental RNAi. After 6 hours of stress, GFP levels were measured using the Biosorter. GFP values were normalized to time of flight. At least 100 animals were analyzed per condition. For RNA isolation, day 1 adults were treated with 10 mM DTT in S Basal buffer containing *E. coli* OP50 for 2 hours and then snap frozen.
Quantitative PCR

For ER stress induction day 1 adults were washed from their plates and transferred to either control or TM containing plates, where they were incubated for 6h. The animals were then washed off using M9 and snap frozen in trizol. Unstressed synchronized animals were collected at day 1 of adult hood for RNA extraction. RNA was prepared using Zymo Research Direct-zol RNA Microprep kit. SYBR green was used to perform quantitative PCR (RT-qPCR). See Supplementary Table 2 for list of qPCR primers used in this study. Primer efficiency was tested and was always above 90%.

Lifespan analysis

Adult lifespan analysis was performed at 20°C on mutant and WT *C. elegans*. The animals were synchronized in a four-hour egg lay. Animals were scored as dead or alive every second day until the end of the experiment. Animals were transferred every day for the first seven days. Statistical analysis on the Kaplan-Meier survival curves was performed using Microsoft Excel. See Supplementary Table 1 for lifespan statistics. Each survival experiment was performed at least twice.

Hydrogen peroxide survival assay

1M hydrogen peroxide (H$_2$O$_2$) was added to unseeded NGM plates to a final concentration of 1 µM and allowed to dry for several minutes. Synchronized day one adults were then transferred onto the plates and incubated at 25°C. The animals were then scored every 2 hours for survival. 20 to 50 animals were used in each experimental condition.

Pathogenic bacteria survival assay

The *Pseudomonas aeruginosa* strain PA14 was grown in LB media and seeded onto high peptone NGM plates and incubated for 12 hours at 37°C immediately before the start of the experiment. Day one animals were first transferred to unseeded NGM
plates to crawl around the plate and remove any excess *E. coli* OP50 off of their bodies. They were then transferred to the PA14 containing plates and incubated at 25°C. The animals were scored every 12 hours until all animals were dead. At least 75 animals were used in each experimental condition.

**Heat stress survival assay**

Day 1 synchronized *C. elegans* were transferred to fresh NGM plates seeded with *E. coli* OP50. These plates were transferred to a 35°C incubator, where they were evenly distributed throughout the incubator to ensure even heat exposure. The plates were scored for live worms every 2 hours until all of the worms were dead. 20 to 50 animals were used in each experimental condition.

**Sample collection and RNA purification for sequencing**

For RNA sequencing we used day one adults that were hatched within one hour. To achieve this synchronization, we washed all adults and larvae of plates with M9 and allowed the eggs on hatch for one hour. The freshly hatched L1 larvae were then washed off and transferred to fresh plates seeded with OP50 and incubated at 20°C until they developed to adults. At day one of adulthood, animals were washed from their plates and transferred to either control or TM containing plates, where they were incubated for 6 hours. The animals were then washed off using M9 and snap frozen in trizol. All biological replicates were collected and prepared using a snaking collection method to reduce batch effects. Total RNA was purified using Zymo Research Direct-zol RNA Microprep kit.

**RNA-seq library preparation**

RNA quality was assessed using Agilent’s Bioanalyzer platform, and samples with RIN > 9 were used for library construction. 2.5 µg of total RNA was subjected to ribosomal RNA (rRNA) depletion using the Illumina’s Ribo-zero Gold kit (Illumina), according to
the manufacturer’s protocol. Strand specific RNA-seq libraries were then constructed using the SMARTer Stranded RNA-Seq HT Kit (Clontech #634839), according to the manufacturer’s protocol. Based on rRNA-depleted input amount, 15 cycles of amplification were performed to generate RNA-seq libraries. Paired-end 150 bp reads were sent for sequencing on the Illumina HiSeq-Xten platform at the Novogene Corporation (USA). The resulting data was then analyzed with a standardized RNA-seq data analysis pipeline (described below).

**RNA-seq analysis pipeline**

cDNA sequences of worm genes were obtained through ENSEMBL Biomart for the WBCel235 build of the *C. elegans* genome (Ensemble release v94; accessed 2018-12-03). Trimmed reads were mapped to this reference using kallisto 0.43.0-1 and the –fr-stranded option (Bray et al., 2016). All subsequent analyses were performed in the R statistical software (https://cran.r-project.org/). Read counts were imported into R, and summarized at the gene level. Differential gene expression was estimated using the ‘DESeq2’ R package (DESeq2 1.16.1) (Love et al., 2014). David analysis (version 6.8) was performed to identify significantly enriched gene ontology terms.

**Developmental verapamil (VPL) resistance assay and lifespan analysis**

Plates containing 10 μg/mL TM were supplemented with 1 nM VPL by adding VPL solved in DMSO onto the plates. Plates were allowed to dry for 6h before eggs were transferred to them to determine developmental resistance to TM. Plates were then scored after 4 days to determine the relative number of L4 larvae or adults. Lifespan assays were performed as above, however the animals were transferred to fresh VPL containing plates every second day for the whole lifespan assay. 20 to 50 animals were used in each experimental condition.
Data availability

Raw sequencing data were deposited to the NCBI Gene Expression Omnibus (GEO) under the accession number GSE144675.

Acknowledgements

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Competing interests

The authors declare no competing interests.
References


Figure Legends

**Figure 1. Dye filling defect**ive C. elegans mutants are resistant to tunicamycin

A. Red fluorescence and differential interference contrast confocal microscopy images of WT and osm-3(dh441)IV C. elegans after DiI treatment.

B. Developmental tunicamycin (TM) resistance assay using the indicated concentrations with WT animals and osm-3(dh441)IV mutants.

C. Demographic lifespan analysis of WT and osm-3(dh441)IV animals. WT mean lifespan = 24 days, osm-3(dh441)IV mean lifespan = 29 days, p<0.0001.

D. Developmental TM resistance assay with WT and daf-10(e1387)V mutants.

E. Developmental TM resistance assay with WT and odr-3(n2150)V mutants.

F. Table of dye filling phenotype and TM resistance. In the dye filling column, + is positive for DiI filling as in Figure 1A and - is Dyf. In the TM resistant column, + is resistant and - is not resistant to 10 µg/mL TM.

**Figure 1 Supplement 1**

A. osm-3(dh441)IV has a C to T mutation at position 3796925 of exon 8.

B – J. Developmental TM resistance assay with WT, odr-1(n1936)X, tax-4(p678)III, che-11(e1810)V, daf-10(e1387)V, osm-1(p808)X, osm-6(p811)V, daf-1(mn335)I, che-2(e1033)X and osm-5(p813)X mutants.

**Figure 2. Tunicamycin-induced ER stress response is blunted in osm-3 mutants**

A. DAVID gene ontology (GO) terms that are enriched in TM-treated WT compared to TM-treated osm-3(dh441)IV worms. Red = upregulated and blue = downregulated.

B. Green fluorescent images of WT and osm-3(dh441)IV animals in the hsp-4::GFP reporter background after 6 hours of 10 µg/mL TM treatment. Worms are outlined in the images.

C. Biosorter analysis of osm-3(dh441)IV vs. WT animals in the hsp-4::GFP background after 6 hours of control or TM treatment. Data are mean +SEM, n=4, *** p<0.0001 by two-way ANOVA.

D. Green fluorescent images of WT and osm-3(dh441)IV animals in the hsp-4::GFP background after development on control or sel-1 RNAi. Worms are outlined in the images.

E. Biosorter analysis of osm-3(dh441)IV vs. WT animals in the hsp-4::GFP background raised on control or sel-1 RNAi. Data are mean +SEM, n=3, ** p<0.005 *** p<0.0001 by two-way ANOVA.

F. Quantitative PCR measuring relative hsp-4 and spliced and unspliced xbp-1 mRNA levels in WT and osm-3(dh441)IV animals after 2 hours of 10 mM DTT treatment. Data are mean +SEM, n=3, * p<0.05, ** p<0.001, *** p<0.0001 by two-way ANOVA.

**Figure 2 Supplement 1**

A. Quantitative PCR measuring relative mRNA expression of indicated genes in WT animals and osm-3(dh441)IV mutants after 6 hours of vehicle or TM treatment.
Figure 3. Tunicamycin resistance in osm-3 mutants is not daf-16 or pmk-1 dependent

A. Demographic lifespan analysis of WT, osm-3(dh441)IV, daf-16(mu86)I and osm-3(dh441)IV; daf-16(mu86)I animals. WT mean lifespan = 22 days, osm-3(dh441)IV mean lifespan = 25 days p<0.005 compared to WT, daf-16(mu86)I mean lifespan = 16 days p<0.0001 compared to WT, osm-3(dh441)IV; daf-16(mu86)I mean lifespan = 16 days p<0.0001 compared to WT.

B. Developmental resistance assay using 10 µg/mL TM with WT, osm-3(dh441) IV, daf-16(mu86) I, and osm-3(dh441) IV; daf-16(mu86) I animals.

C. Developmental resistance assay using 10 µg/mL TM with WT, osm-3(dh441) IV and daf-2(e1370) III animals.

D. Pseudomonas aeruginosa PA14 survival assay with WT, osm-3(dh441) IV, pmk-1(km25) IV, and osm-3(dh441) IV; pmk-1(km25) IV animals. WT mean survival = 44 hours, osm-3(dh441) IV mean survival = 58 hours p<0.001 compared to WT, pmk-1(km25) IV mean survival = 31 hours p<0.001 compared to WT, osm-3(dh441) IV; pmk-1(km25) IV mean survival = 34 hours p<0.001 compared to WT and p=0.06 compared to pmk-1(km25) IV.

E. Developmental resistance assay using 10 µg/mL TM with WT, osm-3(dh441) IV, pmk-1(km25) IV, and osm-3(dh441) IV; pmk-1(km25) IV animals.

Figure 3 Supplement 1

A. Biosorter analysis of osm-3(dh441) IV vs. WT animals in the hsp-4::GFP background raised on control or daf-16 RNAi. Data are mean +SEM, n=4, *** p<0.0001 by two-way ANOVA.
Figure 4. P-glycoprotein inhibition suppresses osm-3 tunicamycin resistance

A. Developmental TM resistance assay using the PGP inhibitor verapamil (VPL) using 10 µg/mL TM supplemented with vehicle or 1 nM VPL. Data are mean ±SD, n=3, *** p < 0.001 by two-way ANOVA.

B. Developmental paraquat resistance assay of WT animals and osm-3(dh441)IV mutants using 0.2 mM paraquat and vehicle control. Data are mean ±SD, n=3, *** p < 0.001 by two-way ANOVA.

C. Developmental ivermectin resistance assay of WT and osm-3(dh441)IV mutants using 6 ng/mL ivermectin and vehicle control. Data are mean ±SD, n=3, *** p < 0.001 by two-way ANOVA.

D. Demographic lifespan analysis on vehicle and VPL treated WT and osm-3(dh441)IV worms. Vehicle treated: WT mean lifespan = 19 days; osm-3(dh441)IV mean lifespan = 24 days p<0.0001 compared to WT vehicle. VPL treated: WT mean lifespan = 19 days; osm-3(dh441)IV mean lifespan = 22 days, p<0.005 compared to WT Vehicle.

Figure 4 Supplement 1

A. Heat shock assay at 35°C in WT and osm-3(dh441)IV animals on day 1 of adulthood. WT mean survival = 7 hours, osm-3(dh441)IV mean survival = 6 hours, p<0.01

B. Hydrogen peroxide survival assay using 1µM H₂O₂ on day 1 of adulthood in WT and osm-3(dh441)IV animals. WT mean survival = 7 hours, osm-3(dh441)IV mean survival = 6 hours, no significant difference

C. WT development on 0 to 1µM Verapamil. No significant difference in development.
Figure 5. *nhr-8* signaling regulates xenobiotic detoxification response through PGPs

A. Quantitative PCR measuring relative PGP mRNA expression in WT, *osm-3(dh441)IV, nhr-8(hd117)IV* and *osm-3(dh441)IV; nhr-8(hd117)IV* animals. Data are mean +SEM, n=3, *p<0.05, **p<0.001, ***p<0.0001 by two-way ANOVA.

B. Developmental TM resistance assay on 10 µg/mL TM and control with WT, *osm-3(dh441)IV, nhr-8(hd117)IV* and *osm-3(dh441)IV; nhr-8(hd117)IV* animals. Data are mean +SD, n=3, *p<0.05, ***p<0.0001 by t-test.

C. Quantitative PCR measuring relative *pgp-8* mRNA expression in WT and *nhr-8* overexpressing (nhr-8 OE) extrachromosomal array dhEx451[coel::rfp;nhr-8::gfp]. Data are mean +SEM, n=3, *p<0.05 by t-test.

D. Developmental dose response TM resistance assay using the indicated TM concentrations with transgenic animals containing an *nhr-8* overexpressing extrachromosomal array dhEx451[coel::rfp;nhr-8::gfp]. “- Ex Array” do not visibly contain the extrachromosomal array as judged by the co-injection marker and “+ Ex Array” contain the extrachromosomal array. Data are mean +SEM, n=3, *p<0.05 and ***p<0.005 by two-way ANOVA. At least 100 animals were used in each condition.

Transmission rates of the extrachromosomal array was assayed on parallel plates.

E. Biosorter analysis of *osm-3(dh441)IV* in the *hsp-4::GFP* background raised on control or *pgp-8* RNAi after 6 hours of control or 20 µg/mL TM treatment. Data are mean +SEM, n=3, *p<0.05, ***p<0.0001 by two-way ANOVA.

Figure 5 Supplement 1

A. Demographic lifespan analysis of WT, *osm-3(dh441)IV, nhr-8(hd117)IV* and *osm-3(dh441)IV; nhr-8(hd117)IV* animals. WT mean lifespan = 22 days, *osm-3(dh441)IV mean lifespan = 14 p<0.0001 compared to WT, *nhr-8(hd117)IV* mean lifespan = 19 days p<0.0001 compared to WT.

Figure 6. Sterol signals modulate TM resistance

A. Developmental dose response TM resistance assay using the indicated TM concentrations with WT animals and *osm-3(dh441)IV* mutants raising on 0 or 5 µg/mL cholesterol.

B. Cholesterol dose response developmental assay with WT, *osm-3(dh441)IV, nhr-8(hd117)IV* and *osm-3(dh441)IV; nhr-8(hd117)IV* animals on vehicle or 5 µg/mL TM.

C. Biosorter analysis of WT animals with the *hsp-4::GFP* reporter raising on 0 or 50 µg/mL cholesterol after 6 hours of vehicle or 5 µg/mL TM treatment. Data are mean +SEM, n=3, *p<0.05, ***p<0.0001 by two-way ANOVA.

D. Quantitative PCR measuring relative *pgp-8* mRNA expression in WT animals raised on 0 or 5 µg/mL cholesterol. Data are mean +SEM, n=3, *p<0.05 by t-test.
Figure 1. Dye filling defective *C. elegans* mutants are resistant to tunicamycin

A. WT

B. Percent Developed to L4/Adult

C. Percent survival

D. Percent Developed to L4/Adult

E. Percent Developed to L4/Adult

F. Dye Filling, Tunicamycin Resistant

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Figure 2. Tunicamycin induced ER stress response is blunted in osm-3

A. GO Term Enrichment

B. No Stress 6 hours TM

C. hsp-4::GFP Intensity

D. Control RNAi sel-1 RNAi

E. hsp-4::GFP Intensity

F. UPR ER Induction

WT osm-3(dh441)IV

Vehicle TM

Vehicle sel-1 RNAi

Vehicle DTT
Figure 3. Tunicamycin resistance in *osm-3* is not *daf-16/IIS* or *pmk-1* dependent.
Figure 4. P-glycoprotein inhibition suppresses osm-3 TM resistance

A. B. D.

C.
Figure 5. nhr-8 signaling regulates xenobiotic detoxification response through PGPs

A. 

B.

Wild type

osm-3(dh441)IV

nhr-8(hd117)IV

osm-3(dh441)IV; nhr-8(hd117)IV

C.

D.

E.

osm-3(dh441)IV

hsp-4::GFP Intensity

pgp-8 RNAi

***

*
Figure 6. Sterol signals modulate TM resistance

A.

B.

C.

D.
Supplementary Figure 1.

A. osm-3
M02B7.2

B. WT
odr-1(n1936)X

C. WT
tax-4(p678)III

D. WT
che-11(e1810)V

e. WT
daf-10(e1387)V

F. WT
osm-1(p808) X

G. WT
osm-6(p811) V

H. WT
daf-1(mn335)I

I. WT
che-2(e1033)X

J. WT
osm-5(p813)X
Supplementary Figure 2.

A.

![Graph showing relative mRNA expression](image-url)

- **hsp-4**
- **cnx-1**
- **crl-1**
- **pdi-1**
- **ero-1**
- **dnj-27**
- **T14G8.3**
- **T24H7.2**

**Legend:**
- Black: Wild type- Vehicle
- Dotted black: Wild type- TM
- Red: osm-3(dh441)IV- Vehicle
- Maroon: osm-3(dh441)IV- TM
Supplementary Figure 3.

A.

**hsp-4::GFP Intensity**

- **Vehicle**
- **TM**

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* *** p < 0.001
** ns = not significant

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Supplementary Figure 4.

A. Survival at 35°C

B. \( \text{H}_2\text{O}_2 \) Survival

C. Wild type development to L4/Adult
Supplementary Figure 5.

A.

![Graph showing percent survival over time for different genotypes.](image)

- Wildtype
- `osm-3(dh441)IV`
- `nhr-8(hd117)IV`
- `osm-3(dh441)IV; nhr-8(hd117)IV`