Particulate Air Pollutants Exacerbate Polyglutamine Aggregation in *C. elegans*

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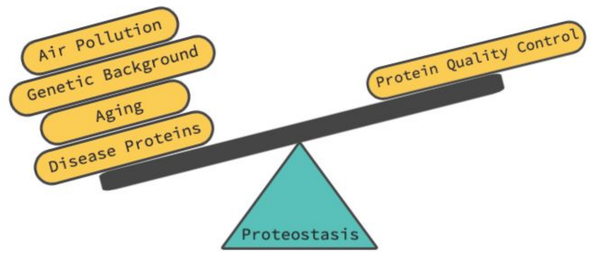
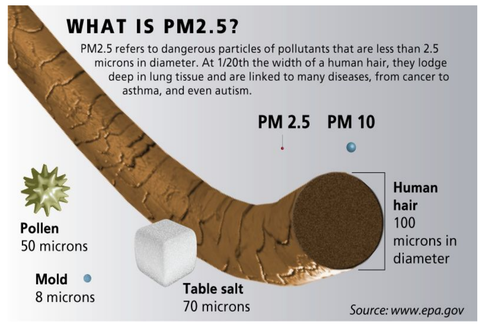
Abstract

The proteostasis network refers to the biochemical pathways that together regulate protein synthesis, transport, folding, and degradation. Functional decline in the proteostasis network activity results in protein misfolding, aggregation, and, often, toxicity. Diseases of protein conformation, such as Alzheimer's disease (AD), Huntington's disease (HD), and Machado-Joseph disease (MJD) seem to have proteostasis collapse at their core. Interestingly, recent studies showed that particulate air pollution may be a significant environmental risk factor for AD. Some studies have suggested that poor air quality, specifically nanoparticulate matter (nPM), provokes the aggregation and toxicity of neurodegenerative disease-associated proteins resulting in cognitive decline in mouse models of AD. We propose that nPM may act through the proteostasis network to indirectly disrupt the folding of disease-associated proteins. We are currently testing the hypothesis that the proteostasis machinery and the folding of neurodegenerative disease-associated proteins, such as huntingtin (htt), and polyQ expansions, expressed in *C. elegans* can be affected by controlled extrinsic factors. To induce proteostasis disruption, we treated wild type animals with sodium azide (Na-Azide), azetidine (AZC), paraquat (PQ), and heat shock, which are all known to disturb protein folding, and determined sub-lethal concentrations for each chemical. In preliminary studies of the effect of these proteostasis-disrupting agents on htt protein folding, we exposed *C. elegans* that have been engineered to express a fragment of the human htt protein in body wall muscle cells. Exposure of animals expressing human htt in body wall muscle cells to 5mM AZC or 5mM Na-Azide resulted in a statistically significant increase in protein aggregation. To investigate the effect of nPM on protein aggregation in *C. elegans*, we exposed polyQ-expressing animals to nPM and measured aggregation. Animals exposed to nPM displayed a statistically significant increase in polyQ aggregation in comparison to unexposed animals. Overall, our study suggests that nano-sized particulate air pollution exacerbates the misfolding of neurodegenerative disease-associated proteins in *C. elegans.* Thus, we propose that proteostasis disruption in response to nPM may be a significant determinant in the onset of neurodegenerative disease.

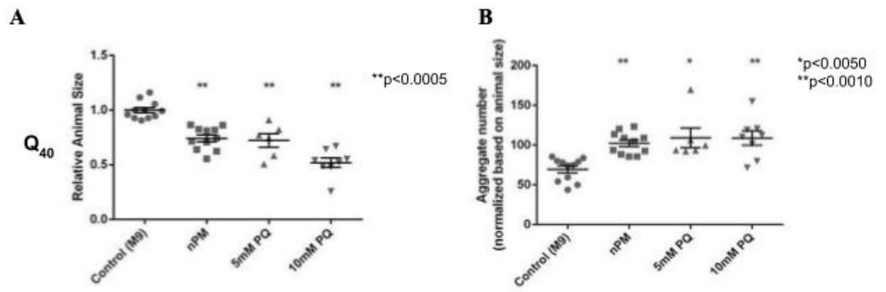
Does air pollution trigger disease by disrupting the proteostasis balance?

We hypothesize that small particulate matter produced by the combustion of fossil fuels may be an important source of proteotoxic stress that contributes to the overall misfolded protein load. The resultant imbalance in proteostasis may be the direct cause of the observed increased risk for disease (1).



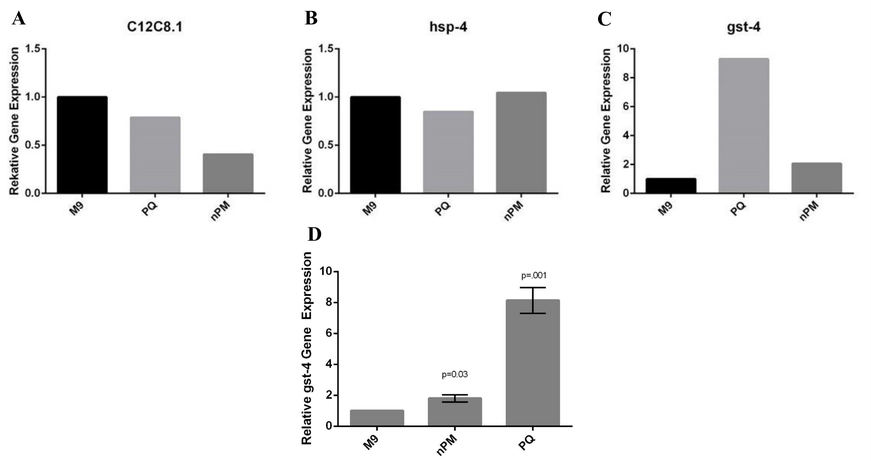


Particulate air pollution has comparable effects on polyglutamine aggregation as paraquat



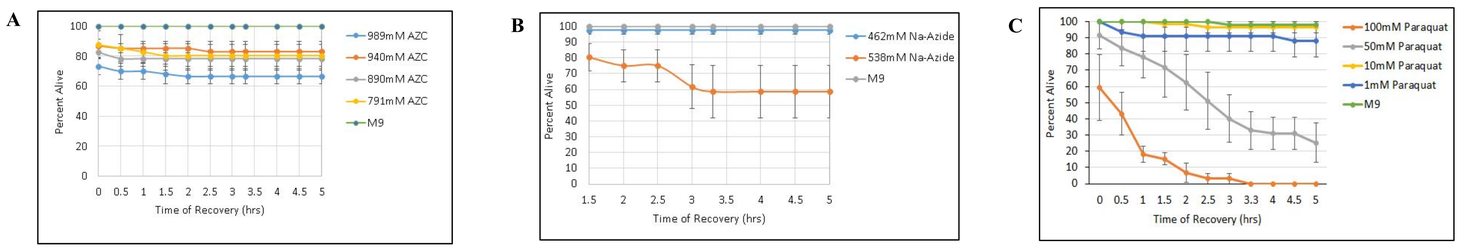
To explore how nPM results in polyQ aggregation, Q_{40} animals were synchronized at L4 larval stage and exposed to M9 (n=11), 75µg/mL nPM (+nPM) (n=11), 5mM PQ (n=6) or 10mM PQ (n=8) from L4 until day three of adulthood. Q_{40} animals were scored for (A) size and (B) aggregate number. Exposure of L4 Q_{40} animals to 75µg/mL nPM, 5mM PQ or 10mM PQ resulted in reduced body size in comparison to control (p-value<0.0005) (A). Animals treated with nPM and 10mM PQ displayed a higher aggregate number in comparison to control animals (p-value<0.0010) (B). Similarly, animals exposed to 5mM PQ had higher aggregate number per unit area than controls (p-value<0.0050) (B).

Chronic exposure to particulate air pollution induces an oxidative stress response



To examine changes in gene expression of C12C8.1 induced by heat shock response (A), hsp-4 induced by the unfolded protein response (B), and gst-4 induced by oxidative stress (C) in response to chronic exposure to nPM and paraquat (PQ), Q35 animals were exposed to nPM or PQ for 3 days at the L4 stage of development. qRT-PCR results from homogenized samples show that paraquat exposure induces the expression of the oxidative stress response gene, gst-4, and animals exposed to nPM have a two-fold increase in gst-4 expression (C). The data for gst-4 was replicated in triplicates (D). Animals exposed to nPM induced a significantly higher gst-4 expression (p-value=0.030) in comparison to control animals (D).

Sub-lethal concentrations of chemicals that disrupt proteostasis were determined for wild type animals

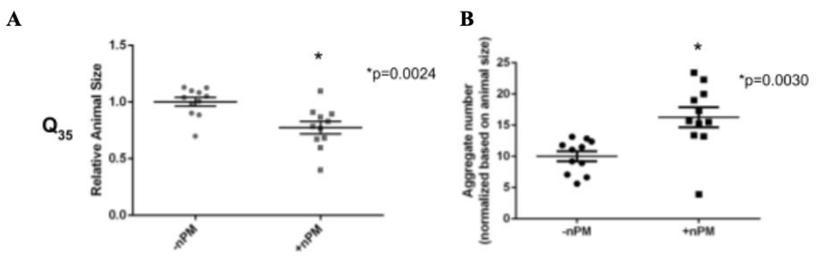


To model proteostasis impairment, we treated animals with varying concentration of azetidine (AZC) (A), sodium azide (Na-Azide) (B), and paraquat (C).

- Azetidine is a proline analogue, which can get incorporated into proteins in the place of proline and induce protein misfolding (3).
- Sodium azide is a toxic chemical often used in agriculture for pest control and known to produce superoxide anions (4).
- Paraquat is a potent herbicide, as it is redox-active, producing superoxide anions (5).

To determine the concentrations that are sub-lethal but toxic to the majority of the *C. elegans* population, we performed survival assays following 1.5hrs of exposure to each chemical. 989mM azetidine, 538mM sodium azide, and 50mM paraquat resulted in a gradual decline in survival over time.

Particulate air pollution exacerbates polyglutamine aggregation



To determine whether nPM disrupts the folding of neurodegenerative disease-associated proteins, we examined the polyglutamine (polyQ) protein aggregation. PolyQ expansions are linked to several neurodegenerative diseases, including HD and MJD, each characterized by expansion of a polyQ-encoding CAG repeat in different proteins. To study the effect of exposure to nPM on polyQ aggregation, we used animals expressing the polyQ length, 35 glutamines (Q_{35}), which is a threshold length for aggregation (6).

24 L4 stage Q_{35} animals were exposed to 75µg/mL nPM (+nPM) and 18 were unexposed (-nPM) from L4 until day three of adulthood. Animals were scored for (A) size and (B) aggregate number. We found no significant difference in the number of aggregates in Q_{35} animals exposed to nPM and control animals that were not exposed to nPM. However, Q_{35} animal size, after exposure as L4s to nPM for three days, was less than that of unexposed control animals (p-value=0.0024) (A). After normalization to size, the number of aggregates per unit area in Q_{35} animals exposed to 75µg/mL nPM was 1.5-fold higher than that of animals that were not exposed to nPM (p-value=0.0030) (B).

Future Directions

- Examine changes in aggregation composition of Htt513, PolyQ, and Ataxin-3 in *C. elegans* in response to nPM exposure
- Examine changes in gene expression in response to exposure to nPM
- Determine if genetic background modulates response to nPM exposure.

References

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