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**Characterizing Transcriptional Activation of the Endoplasmic Reticulum Unfolded Protein
Response in *Caenorhabditis elegans* Models of Tau Pathology**

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Abstract

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To endure over the organismal lifespan, neurons utilize multiple strategies to achieve protein homeostasis (proteostasis). Some homeostatic mechanisms act in a subcellular compartment-specific manner, but others exhibit trans-compartmental mechanisms of proteostasis. Age-associated changes in neuronal proteostasis dynamics occur in neurodegenerative diseases. Under physiological conditions, tau protein in the cytoplasm modulates microtubule dynamics necessary for structural and regulatory functions in neurons. Under pathological conditions, abnormal tau protein species contribute to a group of neurodegenerative diseases known as tau proteinopathies (tauopathies). To identify pathways protecting neurons from pathological tau

protein, I employ a transgenic *Caenorhabditis elegans* (*C. elegans*) model of human tauopathy exhibiting proteostatic disruption. I show normal functioning of the endoplasmic reticulum unfolded protein response (UPR^{ER}) promotes clearance of pathological tau, and loss of the three UPR^{ER} branches differentially affects tauopathy phenotypes. Loss of function of *xbp-1* and *atf-6* genes, the two main UPR^{ER} transcription factors, exacerbates tau toxicity. Furthermore, constitutive activation of master transcription factor XBP-1s ameliorates tauopathy phenotypes. However, both ATF6 and PERK branches of the UPR^{ER} participate in amelioration of tauopathy by constitutively active XBP-1, possibly through endoplasmic reticulum-associated protein degradation (ERAD). Given the importance of UPR^{ER} transcriptional regulation, I conduct transcriptomic analysis to enrich for neuronal transcripts and nominate five XBP-1s target genes with human orthologs and an association with ATF6 (*csp-1*, *dnj-28*, *hsp-4*, *ckb-2*, and *lipl-3*). CSP-1, DNJ-28, HSP-4, and LIPL-3 function are necessary for XBP-1s-mediated tauopathy suppression. Furthermore, functional HSP-4 modulates tauopathy phenotypes. Understanding how the UPR^{ER} modulates pathological tau accumulation will inform neurodegenerative disease mechanisms and directed studies in mammalian systems with the long-term goal of identifying therapeutic targets in human tauopathies.

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After graduating from UW with a Bachelor of Science in Neurobiology, I pursued research experience as a laboratory technician in Dr. Cyrus Zabetian's laboratory at the VA Puget Sound Health Care System (VAPSHCS). This was my first experience conducting research full-time, which prepared me for conducting research as a graduate student. When I decided it was time to apply for graduate school, Dr. Zabetian and Dr. Ignacio Mata were incredibly helpful by allowing me to conduct my own independent research project and supporting me throughout the application process. Although I was just a laboratory technician, I am thankful for your mentorship which facilitated gaining new skills necessary on my path to becoming an independent scientist.

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Dedication

This doctoral dissertation is dedicated to those pursuing their dreams, regardless of obstacles or setbacks. Your journey may change paths along the way, but your potential, drive, and dedication to achieve your goals will always guide you. You have the ability to harness this power, utilize your creativity, and impact the world one step at a time.

“Don't adventures ever have an end? I suppose not. Someone else always has to carry on the story.” — Bilbo Baggins (*The Fellowship of the Ring*)

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CHAPTER I:

Tau protein homeostasis in neuronal health and disease

Summary

The work in this chapter was derived from primary and review literature.

Microtubule-associated protein tau gene

The human microtubule-associated protein tau (*MAPT*) gene is composed of 16 exons numbered 0-14¹. on chromosome 17q21². Exon 1 contains both the 5' untranslated region (UTR) as well as the start codon of the protein, while exon 14 contains the 3' UTR³. The *MAPT* gene promoter includes exon 1, which is transcribed, but not translated. Exons 1, 4, 5, 7, 9, 11, 12, and 13 are present in all major splice isoforms of the tau protein, while exons 2, 3, and 10 are alternatively spliced. The alternative splicing pattern of the *MAPT* gene generates the six major tau protein isoforms found in the human brain⁴. The *MAPT* gene is transcribed into its nuclear ribonucleic acid (RNA) which, after intron splicing and polyadenylation, becomes *MAPT* messenger RNA (mRNA). This transcription unit is a single entity that is translated into tau protein and includes the 5' UTR, the coding DNA sequence (CDS), and the 3' UTR region.

Tau protein

Structure

The six major tau protein isoforms in the human brain are produced via alternative splicing at exons 2, 3, and 10 of the *MAPT* gene. These isoforms, which range from 352-441 amino acid residues, differ in the number of amino (N)-terminal inserts and carboxy (C)-terminal repeats. Structurally, tau protein has three main regions: the acidic N-terminal region (i.e., projection domain), a basic positively-charged proline-rich region in the middle, and the C-terminal region (i.e., microtubule assembly domain). In the N-terminus, exons 2 and 3 encode two 29 amino acid sequences. Tau isoforms can contain zero, one, or two inserts in the projection domain (i.e., 0N, 1N, and 2N, respectively). The N-terminal domain is referred to the projection domain, namely because it projects from the microtubule surface and can interact with other cytoskeletal elements. In the C-terminus, exons 9-12 encode 18 amino acid sequences which are repeated either three or four times. Tau isoforms can contain three or four repeat domains in the microtubule assembly domain (i.e., 3R or 4R, respectively). The second repeat is encoded by exon 10, which is not included in 3R tau isoforms.

Tau protein exhibits several unique biochemical features, which contribute to its overall shape and function. It is highly water soluble and stable under both acidic conditions and high temperatures. Nuclear magnetic resonance and small-angle X-ray scattering imaging revealed tau is natively unfolded and does not readily form aggregates⁵. Despite this, tau preferentially adopts a paperclip-like shape, in which the N-terminal, C-terminal, and repeat domains all approach each other⁶. This paperclip structure may protect tau from aggregation. Overall, tau protein is basic; however, the N-terminal residues are acidic and the C-terminus is approximately

neutral. This difference in charges is important for interactions with other proteins (e.g., microtubules), internal protein folding, and protein aggregation.

Post-translational modifications

Tau protein can undergo a variety of post-translational modifications. Phosphorylation is the most well-studied modification, given its involvement in regulating the physiological functions of tau (e.g., binding to microtubules). There are 85 potential phosphorylation sites (45 serine, 35 threonine, and 5 tyrosine amino acids) in the longest tau isoform (i.e., 2N4R) that are generally accessible due to the natively unfolded structure of tau⁷. The microtubule binding affinity of tau is primarily regulated by serine/threonine-directed phosphorylation. Tau phosphorylation is developmentally regulated; it is much higher in the fetal brain when compared to the adult brain⁷. Tau protein has also been shown to be post-translationally modified via acetylation, glycosylation, glycation, deamidation, isomerization, nitration, methylation, ubiquitination, sumoylation, and truncation⁸. Some of these post-translational modifications may impact tau turnover and normal function (e.g., acetylation, methylation, ubiquitination, and sumoylation), while others may be associated with pathological conditions (e.g., oxidation, glycation, nitration, and truncation)⁹. However, the physiological significance of these tau post-translational modifications has yet to be fully characterized.

Function

Tau was first identified as a microtubule-associated protein. Because tau modulates microtubule dynamics, tau protein is important for structural and regulatory functions in neurons. Neurons are asymmetrical cells with processes extending relatively long distances. In adult neurons, tau is mainly found in the subcellular axonal compartment. One role of the microtubule network is maintaining proper neuronal morphology. Tau can stabilize microtubules, promote microtubule

assembly, and regulate reorganization of the cytoskeleton^{10,11}. Another role of the microtubule network is axonal transport of critical cargoes, such as signaling molecules, trophic factors, organelles, and vesicles. Transportation of cargoes along microtubules is achieved via two motor proteins: dynein (i.e., towards the cell body) and kinesin (i.e., towards the axonal terminus)¹². Under normal physiological conditions, tau is in a constant dynamic equilibrium with microtubules, which is controlled primarily by the phosphorylation state of tau. Phosphorylation via kinases allows tau to bind microtubules, while dephosphorylation via phosphatases allows tau to detach from microtubules. The frequent cycling of bound and unbound tau is necessary for effective axonal transport since tau competes with the motor proteins for microtubule binding.

Overall, the six tau protein isoforms seem to be functionally similar, but the unique expression pattern of these isoforms is likely to be involved in distinct physiological roles throughout aging. Tau isoforms are differentially expressed during development. In the fetal human brain, only the shortest tau isoform is expressed (i.e., 0N3R), referred to as fetal tau^{13,14}. The absence of the second microtubule repeat domain (i.e., 4R) and high level of phosphorylation during fetal development account for enhanced cytoskeletal plasticity required for growing immature neurons¹⁴⁻¹⁷. In most regions of the adult human brain, such as the cerebral cortex, the 3R and 4R tau isoforms are expressed in a one-to-one ratio¹⁸. Given 4R tau isoforms include an additional repeat domain in the microtubule assembly domain, they exhibit higher affinity for microtubules and are more efficient at promoting microtubule assembly¹⁸. However, the function of the two N-terminal inserts in the projection domain is unclear. The N-terminal inserts can influence the attachment or spacing between microtubules and other intracellular components^{19,20}. These N-terminal inserts may also affect the subcellular distribution of tau in neurons, since 0N, 1N, or 2N isoforms display distinct subcellular distributions²¹. Finally, *in vitro* studies have shown the inclusion of one N-terminal insert promotes tau protein aggregation, while the inclusion of two N-terminal inserts impedes tau aggregation²².

Tau proteinopathies

Microtubule-associated protein tau gene mutations

Pathogenic mutations in the *MAPT* gene occur in both exonic and intronic regions. Currently, 112 mutations have been identified in the human *MAPT* gene²³. In general, these mutations cause changes in microtubule interactions and/or the tendency of tau to aggregate and modify the production of tau isoforms. Mutations are classified as missense mutations, which alter the sequence of tau, or splicing mutations, which alter the ratio of the different tau isoforms.

MAPT gene missense mutations can affect the peptide sequence and alternative splicing of tau. *MAPT* gene missense mutations are mostly found in or near the microtubule assembly domain. For example, G272V, N279K, Δ K280, P301L, V337M, and R406W mutations cause reduced microtubule affinity and increased aggregation of tau protein^{24,25}. Therefore, *MAPT* gene missense mutations can contribute to either of the two different pathological events of tau protein: loss of function and toxic gain of function.

MAPT gene splicing mutations can affect the ratio of different tau isoforms, but do not lead to the production of mutant tau protein. The majority of *MAPT* gene splicing mutations are found within the mRNA stem loop present at the end of exon 10 and the beginning of intron 10³. Mutations specifically within the mRNA stem loop tend to promote exon 10 inclusion (i.e., the second repeat domain in the microtubule assembly domain). Because 3R tau isoforms do not include the second repeat, these splicing mutations increase the ratio of 4R tau isoforms. These mutations occur in exon 10 (N279K, L284L, Δ N296, N296N, N296H, S305N, and S305S)^{26,27} and intron 10 (E10 + 3, E10 + 14, E10 + 16, E10 + 29)²⁷. Mutations in exon 10 likely cause disruption of the mRNA stem loop or through splicing enhancers and silencers^{4,28}. However, there are also mutations that

decrease the inclusion of exon 10, leading to a reduction in the ratio of 4R tau isoforms. These mutations include Δ K280, L266V, and G272V²⁹. This imbalance of 3R-to-4R tau isoforms leads to tau aggregation; however, the molecular mechanism of how perturbed tau splicing ratios leads to pathology remains unknown.

Pathology

Under pathological conditions, the equilibrium of bound and unbound tau to microtubules is disturbed, leading to an increase in unbound hyperphosphorylated tau. This Dysregulation of physiological tau function triggers a pathological cascade leading to the formation of different abnormal tau protein species in the cytoplasm, ultimately resulting in neurodegeneration. Abnormal disengagement of tau protein from microtubules can be generated by different events, such as an imbalance of tau kinases and/or phosphatases, *MAPT* gene mutations, and other modifications causing and/or promoting tau misfolding. When the amount of unbound tau found in the cytoplasm is increased, tau protein is likely to adopt misfolded conformations. Pretangles are the earliest form of tau deposits that do not exhibit pleated beta-sheet (β -sheet) structures. These pretangles eventually undergo a structural rearrangement to form more organized tau aggregates known as paired helical filaments (PHFs) in which tau monomers have adopted a pleated β -sheet structure and polymerized. Finally, PHFs undergo further self-assembly, producing neurofibrillary tangles (NFTs) and neuropil threads (NTs). In neurons, NFTs are found in the somatodendritic compartment, whereas NTs are localized in distal axons and dendrites. NFTs were initially thought to be the primary toxic species leading to neurodegeneration³⁰; however, increasing evidence indicates intermediate abnormal tau species may confer toxicity³¹. Abnormal tau aggregates are also observed in glial cells in the form of tangles in astrocytes and/or oligodendrocytes. Although it is thought abnormal tau deposition in neurons occurs following the specific steps outlined above, less is known about the progression of tau pathology in glial cells.

The path to tau-mediated neurotoxicity is proposed to be the result of both loss of function and toxic gain of function. Hyperphosphorylated tau in the cytoplasm leads to interruption of microtubule binding and subsequent loss of microtubule stabilization. This tau loss of function affects normal structural and regulatory functions of the cytoskeleton, compromising axonal transport and contributing to synaptic dysfunction and neurodegeneration^{32,33}. Increased unbound tau to microtubules leads to the formation of NFTs in neurons and glial tangles in astrocytes and/or oligodendrocytes. Studies have shown the total level of NFTs correlates with the degree of cognitive impairment^{34,35}, suggesting evidence of a toxic tau gain of function. It is plausible the toxic effects of large NFTs may be due to a direct physical disruption to important cellular processes such as axonal transport²⁶. NFTs may also contribute to tau loss of function by further sequestering normal tau into inclusions. To date, it has been experimentally difficult to discern the contribution of toxic gain of function versus loss of function during the time course of tau-mediated pathogenesis.

The underlying factors determining the spatio-temporal distribution and spreading of tauopathy are currently a matter of debate. These proposed factors include neuronal vulnerability (e.g., poor myelination, high metabolic rates that may confer susceptibility to chronic oxidative stress, and vulnerability to toxins), neuronal and regional connectivity, and processes independent of tau transmission (e.g., spread of neuroinflammation)²⁹. The idea that tau is transmitted between neurons coincides with emerging evidence supporting the “neuronal connectivity” theory of pathological protein spreading²⁹. Although trans-synaptic spreading of tau is a plausible interpretation, the propagation of tau pathology may occur via mechanisms other than the direct transfer of tau between synaptically connected neurons. Further testing is required to validate the clinical significance of the proposed pathological tau propagation mechanisms.

Diseases

Tau represents the most common misfolded protein in human neurodegenerative diseases. Tau proteinopathies (tauopathies) are a heterogeneous group of diseases defined by the presence of abnormal filamentous tau deposits in the brain. Currently, over 30 tauopathies have been identified in humans (**Table 1.1**)³⁶⁻³⁸. These diseases all share some hallmark neuropathological features, such as tau lesions, gross brain atrophy, nerve cell loss, gliosis, superficial spongiosis, and ballooned neurons. However, the identification of distinct tauopathies indicates the overall presence of abnormal tau protein does not affect the brain in a similar fashion. Distinct tauopathies are classified by several neuropathological features (e.g., tau isoforms present in deposits, morphology of inclusions, affected cell types, and affected brain regions)³⁹ and clinical presentation of symptoms. Tauopathies are also classified as sporadic or familial, depending on inheritance of genetic mutations, as well as primary or secondary, depending on the presence of other pathological proteins.

Primary tauopathies are defined as diseases in which tau inclusions are the predominant pathology. Argyrophilic grain disease (AGD), progressive supranuclear palsy (PSP), corticobasal degeneration (CBD), and Pick's disease (PiD) are the most common sporadic primary tauopathies. The tau inclusions found in AGD, PSP, and CBD patient brains are composed of 4R tau isoforms, while the tau lesions found in PiD patient brains are composed of 3R tau isoforms⁴⁰⁻⁴⁵. The hallmark lesions of AGD include argyrophilic grains in neuronal processes and pretangles in neurons, as well as glial tau deposits in astrocytes (known as thorn-shaped astrocytes) and oligodendrocytes^{46,47}. In PSP, neuronal lesions are seen as globose-type NFTs and NTs⁴⁸, while glial lesions are defined as tufted astrocytes and oligodendroglial coiled bodies^{49,50}. The hallmark lesions of CBD appear as intracytoplasmic NTs, pretangles, or small NFTs in neurons, as well as plaques in astrocytes and coiled bodies in oligodendrocytes⁵¹⁻⁵³. PiD is classified by the presence

of tau deposits in neurons known as Pick bodies and reactive astrocytes containing hyperphosphorylated tau lacking a specific name⁵⁴.

Based on the anatomically affected brain regions, AGD, PSP, CBD, and PiD patients primarily exhibit cognitive, movement, and/or behavioral disturbances. In AGD, the limbic system is predominantly affected in patients and clinical presentation of symptoms includes personality changes, emotional imbalance, and mild cognitive impairment⁵⁵. The affected brain regions in PSP patients include the cortex, basal ganglia, and subthalamic nucleus⁵⁶. In CBD patient brains, tauopathy is observed in the hippocampus, basal ganglia, brainstem, and cerebellum⁵⁶. For both PSP and CBD, patients mainly exhibit movement and cognitive problems³⁸. PiD is classified by the presence of tauopathy in neocortical, hippocampal, and subcortical brain regions⁵⁷. PiD is also classified as frontotemporal dementia (FTD) or frontotemporal lobar degeneration (FTLD)⁵⁸. As observed in FTD patients, those with PiD display changes in emotions, behavior, personality, and language⁵⁸.

While most primary tauopathies are classified as sporadic, the identification of mutations in the *MAPT* gene provides a direct link of tau dysfunction with the pathogenesis of disease and clinical manifestation of impairments. In 1998, the first mutations in the *MAPT* gene on chromosome 17q21 were reported in families with an autosomal dominant form of frontotemporal dementia with parkinsonism, which is now known as FTDP-17⁵⁹. Depending on the *MAPT* mutation, tau inclusions found in FTDP-17 brains are composed of 3R tau isoforms, 4R tau isoforms, or a combination of both^{37,60-63}. Neuropathologically, FTDP-17 cases display severe neuronal loss, astrocytic gliosis, and spongiosis, with tau inclusions in neurons or in both neurons and glial cells^{37,38}. Patients with *MAPT* mutations can manifest clinical phenotypes and hallmark pathology similar or identical to those with AGD, PSP, CBD, and PiD³⁸. Additionally, different clinical

phenotypic presentation may be observed in family members carrying the same *MAPT* mutations, indicative of intrafamilial variability⁶⁴.

The most common tauopathy and cause of dementia is Alzheimer's disease (AD), which is classified as a secondary tauopathy due to the presence of another abnormal protein species. In AD, the hallmark lesions include intracellular NFTs and extracellular accumulation of amyloid beta (A β) protein fragments, known as amyloid plaques⁶⁵⁻⁷³. In 1907, Alois Alzheimer and Oskar Fischer first described an association between the clinical presentation of dementia and the postmortem presence of amyloid plaques and NFTs visualized with silver staining in the cerebral cortex⁷⁴⁻⁷⁷. In AD patient brains, both 3R and 4R tau isoforms are present in neuronal tau inclusions^{37,70,78}, which occur in the form of NFTs and NTs. While AD predominantly displays neuronal tauopathy, most other tauopathies described above are characterized by the presence of both neuronal and glial tauopathies. However, in addition to AGD, glial tau deposits known as thorn-shaped astrocytes are common in the elderly with and without AD-related pathology^{79,80}.

Definitive diagnosis of AD requires both clinical history of dementia and neuropathologic confirmation at autopsy. The AD-related pathological process spans decades, and during this time, the distribution pattern of tauopathy lesions develops according to a predictable sequence as determined by postmortem analysis of human brain tissue. Tau inclusions propagate through the brain in a spatio-temporal fashion, providing the basis for reliable disease staging. This differential distribution of tauopathy in the brain is classified using the six Braak stages of AD⁸¹. First, misfolded hyperphosphorylated tau accumulates in the locus coeruleus. During Braak stages I and II, NFTs are present in the transentorhinal and entorhinal cortex and are not associated with clinical symptoms. Braak stages III and IV are defined by greater involvement of the transentorhinal and entorhinal cortex and formation of NFTs in the hippocampus; these stages are associated with the appearance of clinical symptoms, such as mild cognitive impairment. In

Braak stages V and VI, there is extensive spreading of NFTs throughout the cortex, such as in neocortical association areas, and further increase in tauopathy in the brain regions affected during Braak stages I-IV. Patients with Braak stages V and VI are severely demented and meet the neuropathological criteria of AD.

Given AD is a secondary tauopathy with the presence of two pathological proteins, there has been much debate regarding which abnormal protein is the driver of this disease. The amyloid cascade hypothesis was first postulated in 1992 by Hardy and Higgins, who suggested that the accumulation of abnormal A β peptides is the central event in the pathogenesis of AD⁸². AD has been proposed to arise only when A β inclusions have formed separately from and driven the formation of tau inclusions in the neocortex⁸³. However, it has been shown A β accumulation and deposition do not correlate with neuronal loss and cognitive decline. When assessing A β plaque burden via positron emission tomography (PET) scan imaging, there was no association with symptoms of memory impairment in individuals displaying significant plaque burden⁸⁴⁻⁸⁹. Additionally, tau inclusions appear at a younger age than A β deposits^{81,90}. Sparse tau aggregates have been documented in brains from the general population who are younger than 30 years of age³⁸. As described above for the Braak staging of AD, spatio-temporal distribution of NFTs have a stronger correlation than A β plaques with the clinical presentation of disease.

Most AD cases are sporadic, with aging being the greatest risk factor for developing this disease. The majority of patients diagnosed with AD are 65 and older³⁸. However, in 2013, identification of the F176L mutation in the human presenilin 1 (*PSEN1*) gene was found in Alzheimer's first patient, Auguste Deter, whose age of disease onset was 51 years⁹¹. Early-onset AD is rare, and clinical presentation of disease typically occurs in patients in their 30s to mid-60s. The three genes associated with early-onset AD include amyloid precursor protein (*APP*) on chromosome 21, *PSEN1* on chromosome 14, and presenilin 2 (*PSEN2*) on chromosome 1⁹². Mutations in these

genes result in abnormal A β protein production, specifically increased A β ₁₋₄₂ protein fragment production^{92,93}. Other mutations in the *APP* gene can promote the aggregation of A β ₁₋₄₂ protein fragment^{92,93}.

More recently, environmental causes have also been associated with the development of tauopathies. Traumatic brain injury (TBI) occurs from blast injuries, concussive injuries, or both. Moderate and severe TBIs show a dose-response trend as risk factors for neurodegenerative diseases such as cerebral atrophy^{94,95}, AD⁹⁶, chronic traumatic encephalopathy (CTE)⁹⁷⁻¹⁰¹, and Parkinson's disease (PD)¹⁰²⁻¹⁰⁴. TBI and AD share similar pathological hallmarks (i.e., accumulation of A β and hyperphosphorylated tau aggregates) and clinical presentation of dementia. CTE results from repetitive mild TBI and is defined by progressive neurodegeneration with hyperphosphorylated tau aggregates and NFTs. This disease was originally described as the clinical deterioration that occurs after repetitive brain trauma in boxers^{38,105}. CTE patients present with mood, behavioral, and cognitive disturbances. Comprehensive analysis of postmortem brains from individuals who experienced repetitive mild TBI revealed a high frequency of CTE with pathological changes distinct from other neurodegenerative diseases, including AD⁹⁹. Although the underlying mechanisms connecting TBI to these neurodegenerative diseases remain unknown, tauopathy is the common pathological finding.

Tau proteinopathy model systems

To understand underlying mechanisms and develop therapeutics, model systems have been employed for studying human tauopathies. These include single-celled organisms [e.g., *Saccharomyces cerevisiae* (*S. cerevisiae*)], invertebrate models [e.g., *Caenorhabditis elegans* (*C. elegans*) and *Drosophila melanogaster* (*D. melanogaster*)], and mammalian models (e.g., rodents and human cell cultures). Each model system has both advantages and limitations in

recapitulating aspects of human tauopathies, which develop over decades and affect higher cognitive functions. However, each model system is capable of reproducing different features of tauopathy and is instrumental in dissecting the human diseases.

Caenorhabditis elegans animal models

Since its introduction in 1974 by Sydney Brenner, *C. elegans* has proved to be a popular and useful model system for the study of basic biology¹⁰⁶. Many biological fields have taken advantage of this free-living soil nematode, such as genomics, cell biology, neurobiology, and aging. Some of the hallmark features of *C. elegans* as a robust model organism include: short reproductive life cycle (i.e., it reaches adulthood in three days), short lifespan (i.e., approximately two-three weeks), ease of culturing large numbers of animals in a relatively small space (i.e., adults reach approximately one millimeter in length and about 300 progenies originate from one self-fertilized hermaphrodite), and a transparent body (i.e., fluorescent markers can be used for visualization *in vivo*)¹⁰⁶.

C. elegans simplistic and well-characterized nervous system with stereotypical behavioral phenotypes make this an ideal model system for studying neurodegenerative diseases. While the mammalian nervous system consists of billions of neurons, the adult *C. elegans* hermaphrodite has only 302 neurons that reduces the complexity and increases the accuracy of neuronal analyses¹⁰⁷. Additionally, complete neuronal lineage and all synaptic connections have been mapped^{108,109}.

One of the most powerful advantages of using *C. elegans* as a model system is the high degree of genetic conservation with humans and many genetic tools available. Despite the lack of evolutionary complexity, *C. elegans* shares many conserved molecular pathways and cellular mechanisms with mammals, allowing for comparative studies. The OrthoDisease database is

based on 2,466 human disease genes and has predicted more than 500 orthologs in *C. elegans*¹¹⁰. Because genome annotation in both *C. elegans* and humans is an ongoing process, an up-to-date compendium of orthologous genes known as OrthoList 2 was generated¹¹¹. *C. elegans* was the first multicellular organism to have the complete genome sequenced, which resulted in several online databases and resources available for the scientific community^{112,113}. The *C. elegans* genome is relatively small, containing approximately 97 million base pairs, and encodes roughly 20,000 genes¹¹⁴. However, approximately 80% of *C. elegans* genes have clear homologs in the human genome^{115,116}. Genetic screens are commonly utilized in *C. elegans*, such as identifying gene(s) responsible for a particular phenotype (i.e., forward genetics) and analyzing specific phenotype(s) following the disruption of a known gene (i.e., reverse genetics). The two major forward genetic screens employed induce random mutations in the genome by using chemical mutagens (e.g., ethyl methane sulfate) and genome-wide ribonucleic acid interference (RNAi) knockdown of gene transcripts by feeding animals *Escherichia coli* (*E. coli*) expressing a library of double-stranded RNA. Reverse genetics rely on previously generated genetic mutants, RNAi-mediated knockdown of specific genes, and clustered regularly interspaced short palindromic repeats-CRISPR associated protein 9 (CRISPR-Cas9) gene editing technology¹¹⁷.

In *C. elegans*, the protein with tau-like repeats (*ptl-1*) gene is the only gene encoding a protein similar to tau in humans. Like the human *MAPT* gene, *ptl-1* undergoes alternative splicing to produce only two isoforms, PTL-1A and PTL-1B, which contain five and four repeats of approximately 31 residues, respectively¹¹⁸. The PTL-1 protein sequence is about 50% homologous to mammalian tau protein, predominantly in the C-terminal repeat region (i.e., microtubule assembly domain). *C. elegans* PTL-1 protein exhibits biochemical properties comparable to mammalian tau protein, such as thermostability, hydrophobicity, size, amino acid content, charge distribution, predicted secondary structure, and flexibility¹¹⁹. Additionally, PTL-1 protein contains numerous potential glycosylation and phosphorylation sites¹¹⁸⁻¹²⁰. Expression of

ptl-1 is developmentally regulated; postembryonic expression is mainly found in mechanosensory neurons¹²⁰. Functionally, *ptl-1* is involved in egg hatching, touch responsiveness, maintenance of neuronal integrity, and the regulation of lifespan^{121,122}. Following transient damage induced by mechanical stress, PTL-1 was shown to play a role in the maintenance and repair of the microtubule cytoskeleton¹²³. Additionally, PTL-1 physically interacts with Kinesin-3/UNC-104, which is a major motor protein necessary for transporting synaptic vesicles and dense core vesicles in *C. elegans*¹²⁴.

Specific to human neurodegenerative diseases, *C. elegans* is amenable to generating transgenic models. Using DNA transformation and microinjection techniques^{125,126}, human genes encoding proteins involved in neurodegenerative diseases along with a gene encoding a fluorescent protein for monitoring transgenic expression can be introduced into the *C. elegans* genome using cell type-specific promoters. Transgenic *C. elegans* models of human neurodegenerative diseases have been generated for amyotrophic lateral sclerosis (ALS), tauopathies (including AD and FTD/FTLD), PD, and Huntington's disease (HD)¹²⁷. Common *C. elegans* promoters for modeling human neurodegenerative diseases include pan-neuronal, body wall muscle cells, motor neurons, glutamatergic neurons, dopaminergic neurons, touch neurons, and sensory neurons¹²⁷. Exogenous DNA constructs are delivered via a microinjection needle into the gonad of an adult hermaphrodite, which is taken up into the mature oocytes' nucleus. If successful, this will generate a subset of transgenic animals among their progeny expressed as an extrachromosomal DNA array. To overcome low-transmission problems and the consequent gradual loss of extrachromosomal DNA arrays, stable transgenic expression can be generated via chromosomal integration using mutagenic chemicals or irradiation. Stable transgenic lines are further assessed for expression levels of the integrated human gene and phenotypically characterized based on the *C. elegans* promoters used.

C. elegans researchers have developed a variety of assays instrumental for phenotyping transgenic models of human neurodegenerative diseases. Common neurobehavioral assays include the liquid thrashing assay for measuring general neuronal dysfunction, the aldicarb assay for measuring motor neuron dysfunction, the basal slowing response assay for measuring dopaminergic neuron dysfunction, and the mechanosensory assay for measuring glutamatergic neuron dysfunction¹²⁷. Because *C. elegans* are optically transparent, neuronal integrity due to the expression of pathological proteins can be assessed in live animals expressing fluorescent proteins in the neuronal class of interest. Different neuronal health characteristics, such as cell body rounding, disappearance of axons and/or axonal blebbing, broken neurites and/or retraction of dendritic terminals, and neuronal loss can be reliably scored¹²⁷. Finally, given aging is the main risk factor for multiple neurodegenerative diseases, measuring the overall lifespan and/or healthspan is commonly evaluated in transgenic *C. elegans* models. Lifespan is examined using the cohort survival assay¹²⁸ in which animals are scored for survival/death every 24 hours in order to generate a survival curve graph. Similar to humans, *C. elegans* exhibit a decline in physical ability and stress recovery with age. Common healthspan assays include measuring the rate of age-dependent decline of distance moved, thrashing in a liquid, heat stress resistance, and oxidative stress resistance¹²⁹.

Various *C. elegans* transgenic models of human tauopathy have been developed using complementary DNA (cDNA) encoding different human tau isoforms with and without mutations (**Table 1.2**). The first *C. elegans* transgenic tauopathy models were developed in our laboratory and studied FTDP-17 mutations by utilizing a pan-neuronal promoter to overexpress the human 1N4R tau isoform in three forms: wildtype tau, P301L mutant tau, and V337M mutant tau¹³⁰. Our laboratory chose to investigate the human 1N4R isoform because it is the most abundant isoform found in the adult human brain. Both mutant tau transgenic models displayed more severe tauopathy phenotypes when compared to the wildtype tau model, including uncoordinated

movement, accumulation of insoluble phosphorylated tau protein, neuronal loss, and shortened lifespan¹³⁰. However, the V337M mutant tau line displayed the strongest tauopathy phenotypes and was used for further investigation, which will be referred to as Tau (mutant) transgenic *C. elegans*.

Because most human tauopathies are sporadic, reliable wildtype tau transgenic *C. elegans* models were also generated and phenotypically characterized^{131,132}. Previously, our laboratory expressed the human 1N4R tau isoform under a pan-neuronal promoter. After integration, a stable line expressing high levels of wildtype tau was obtained, which will be referred to as Tau (high) transgenic *C. elegans*. Tau (high) animals exhibit severe tauopathy phenotypes, including uncoordinated movement, accumulation of insoluble phosphorylated tau protein, neuronal loss, and shortened lifespan^{131,132}. When compared to Tau (mutant) animals, Tau (high) animals display phenotypes that are equivalent or more severe, allowing the study of strong tauopathy phenotypes driven by expression of human wildtype tau. This type of *C. elegans* tauopathy model is important for exploring potential suppressors of tauopathy phenotypes. Conversely, our laboratory also developed two independent *C. elegans* tauopathy models expressing low levels of the human 1N4R tau isoform after integration. The same pan-neuronal promoter for tau expression was used with a promoter encoding two different fluorescent proteins for monitoring transgenic expression; these will be referred to as Tau (low) A and Tau (low) B transgenic *C. elegans*. Both tauopathy models exhibit mild phenotypes, including uncoordinated movement, neuronal abnormalities, and neuronal loss^{131,132}. This type of *C. elegans* tauopathy model is instrumental for studying potential enhancers of tauopathy phenotypes.

Neuronal protein homeostasis

Proteins, which are the essential building blocks and catalytic machines of all cells, have a finite lifetime and can be prone to misfolding. Depending on the subcellular compartment, there are specific protein quality control mechanisms which respond to the presence of abnormal proteins, such as the cytoplasmic heat shock response, the endoplasmic reticulum (ER) unfolded protein response (UPR^{ER}) with subsequent ER-associated degradation (ERAD), and the mitochondrial unfolded protein response (UPR^{mt}). Once detected, multiple cytoplasmic quality control systems exist to degrade unnecessary or damaged proteins via proteolysis in order to maintain protein homeostasis (proteostasis), including the ubiquitin-proteasome system (UPS), chaperone-mediated autophagy (CMA), and macroautophagy, which will be referred to as autophagy¹³³. The UPS is a proteolytic system in which soluble misfolded proteins are tagged with ubiquitin, unfolded into unstructured polypeptide chains, and cleaved into short peptides while passing through the proteasome¹³³⁻¹³⁶. CMA is responsible for degradation of soluble misfolded proteins that have the KFERQ amino acid sequence degradation signal; this motif is recognized by the chaperone heat shock cognate 70 (Hsc70), and substrates are transported to lysosomes for degradation into amino acids via acidic lysosomal hydrolases^{137,138}. If misfolded proteins are not detected by the UPS and CMA or are prone to forming aggregates, they are directed to autophagy, a bulk degradation system in which substrates are sequestered within double-membraned structures called autophagosomes that eventually fuse with lysosomes for degradation into amino acids via lysosomal hydrolases^{139,140}.

Neurons are particularly vulnerable to challenges maintaining protein quality control over the lifetime. Post-mitotic neurons are more susceptible to the accumulation of cytotoxic proteins, since toxic substances cannot be diluted via cell division¹⁴¹. Given the unique morphology of neurons, protein aggregates in the extensive dendritic processes and long axons need to be isolated inside autophagosomes for retrograde transport to the cell body where there is an abundance of

lysosomes for degradation^{142,143}. Throughout the course of aging, maintaining neuronal proteostasis becomes increasingly difficult. Components of the UPS, CMA, and autophagy in neurons are shown to be downregulated in both expression and activity¹⁴⁴. Post-translational modification (e.g., hyperphosphorylated tau protein) or endoproteolytic cleavage (e.g., A β peptides) of neurodegenerative disease-associated proteins can lead to spontaneous misfolding and aggregation into oligomers enriched in β -sheet structure^{31,145-147}. Genetic mutations in disease-associated proteins can also disrupt their proper folding, leading to the formation of β -sheet aggregates¹⁴⁴. These oligomers are partially resistant to proteolytic degradation pathways and can subsequently form inclusion bodies or extracellular plaques which have highly ordered fibrillar structures with large β -sheet content¹⁴⁵. Cytotoxicity and neuronal death resulting from misfolded oligomers and aggregates is a common molecular mechanism underlying the pathogenesis of many neurodegenerative diseases¹⁴⁸.

Degradation of tau protein occurs via the UPS and autophagy depending on the nature of post-translational modifications influencing folding and solubility. Tau protein can be post-translationally cleaved via endoproteolytic enzymes such as caspases¹⁴⁹, calpain¹⁵⁰, aminopeptidases¹⁵¹, and thrombin¹⁵². However, tau cleavage is unlikely to contribute to the clearance of NFTs since fibrillar tau is protease resistant¹⁵³. These cleavage products with additional post-translational modifications may also contribute to the pathogenesis of tauopathies¹⁴⁴. In general, soluble monomeric tau protein is detected by molecular chaperones and translocated into the 20S proteasome without ubiquitination for degradation. The E3 ligase carboxy-terminus of Hsc70 interacting protein (CHIP) mediates the ubiquitination of tau species (primarily phosphorylated tau) with the assistance of molecular chaperone heat shock proteins (Hsp) Hsp70 and Hsp90 for translocation into the 26S proteasome for degradation¹⁵⁴. The UPS-dependent degradation of tau is facilitated by overexpressing Hsp70¹⁵⁴. However, it remains unclear the extent ubiquitinated tau proteins are ultimately degraded by the proteasome; NFT

deposition has been shown to include not only ubiquitinated tau, but also CHIP and other ubiquitinated proteins^{154,155}. Ubiquitinated tau species can also be delivered to the p62 autophagy adaptor protein for subsequent lysosomal degradation. Tau protein species prone to aggregate formation (e.g., hyperphosphorylated tau) can be cleared via autophagy. Importantly, defective autophagic flux can facilitate the formation of tau oligomers and insoluble aggregates¹⁴⁴.

Endoplasmic reticulum unfolded protein response

Function

Within the eukaryotic cell, the ER is a dynamic membrane network essential for many cellular functions via post-transcriptional modification and proper folding of newly synthesized membrane or secretory proteins. Proper folding of all secretory proteins occurs within the ER, comprising approximately 25% of all proteins in the mammalian proteome^{156,157}. The accumulation of misfolded proteins in the ER, known as ER stress, induces the UPR^{ER} in order to maintain proteostasis via translational attenuation, refolding of misfolded/unfolded proteins, and protein degradation^{158,159}. Protein degradation is achieved through two pathways: ERAD and ER stress-induced pre-emptive quality control (ERpQC). The novel ERpQC pathway degrades newly synthesized ER-targeting proteins in the cytoplasm before their translocation into the ER¹⁶⁰⁻¹⁶². ERAD and ERpQC facilitate degradation of each target protein through the UPS¹⁶³. If the ER stress causes an overloaded protein folding capacity in the ER, the UPR^{ER} triggers caspase-dependent apoptosis to remove stressed cells and maintain tissue homeostasis¹⁶⁴.

The UPR^{ER} is an evolutionarily conserved adaptive mechanism utilized in eukaryotic cells to restore ER homeostasis. In mammals, the UPR^{ER} is comprised of three parallel ER stress sensing

and transducing branches: inositol-requiring enzyme 1 α (IRE1 α), protein kinase RNA-like ER kinase (PERK), and activating transcription factor 6 (ATF6) (**Figure 1.1**; obtained with permission from *Nature Communications*)¹³². The three ER stress sensors embedded in the ER membrane contain a luminal sensor domain and cytoplasmic effector domain, allowing for proper identification of stress within the ER lumen and initiation of transcriptional and translational responses in the cytoplasm. Under homeostatic conditions, the protein chaperone binding immunoglobulin protein (BiP), the ER resident Hsp70 family member, is bound to the luminal sensor domains which holds the ER stress sensors in an inactive conformation. In the presence of misfolded proteins, BiP dissociates from the luminal domain of the three ER stress sensors, allowing for active conformation and stress signal transduction.

IRE1 α (IRE-1 in *C. elegans*) is the most highly conserved and ancient UPR^{ER} transducer, present in both yeast and mammals^{165,166}. In *S. cerevisiae*, Ire1 was identified as the sole ER stress transducer^{167,168}, indicating its overall importance in ER proteostasis. IRE1 α becomes active via the formation of dimers and higher-order oligomers, leading to trans-autophosphorylation¹⁶⁹. Activated IRE1 α catalyzes non-canonical mRNA splicing of a 26 base pair intron from transcription factor X-box binding protein 1 (*XBP1* in mammals; *xbp-1* in *C. elegans*) via its endoribonuclease activity¹⁷⁰. This excision and re-ligation of *XBP1* mRNA shifts the open reading frame, causing translation of the spliced *XBP1* mRNA known as *XBP1s* (*xbp-1s* in *C. elegans*) that is more active and stable than the unspliced *XBP1* mRNA^{171,172}. The *XBP1s* transcription factor translocates to the nucleus and initiates the transcription of cytoprotective genes involved in protein folding, translocation, and glycosylation; redox metabolism; autophagy; cell wall synthesis; vesicular trafficking; ERAD; and lipid/inositol metabolism^{164,167,173,174}.

Although the main role of IRE1 α is upregulating important *XBP1s* transcriptional targets necessary for restoring ER proteostasis, additional activities of this ER stress transducer exist.

Activated IRE1 α promotes degradation of mRNAs and microRNAs encoding ER resident proteins to attenuate protein load entering the ER via a process called regulated IRE1-dependent decay (RIDD)¹⁷⁵⁻¹⁷⁸, which has currently not been found to occur in *C. elegans*¹⁶⁸. In addition, activated IRE1 α induces the activation of c-Jun N-terminal kinase (JNK) through the complex formation with tumor necrosis associated factor 2 (TRAF2) and apoptosis signaling-regulating kinase 1 (ASK1) on the ER membrane, leading to apoptosis¹⁷⁹. Finally, independent of its endoribonuclease domain, IRE α can also initiate ERAD, which is conserved from yeast to mammals, including *C. elegans*¹⁸⁰.

In multicellular eukaryotes, the UPR^{ER} is more complex and contains additional ER stress transducers such as PERK (PEK-1 in *C. elegans*), which is mainly responsible for reprogramming protein translation. Similar to IRE1 α , PERK becomes active after formation of dimers and subsequent trans-autophosphorylation¹⁸¹. Active PERK phosphorylates the eukaryotic translation initiation factor-2 α (eIF2 α) to transiently attenuate global protein synthesis, thereby reducing additional ER protein load^{182,183}. Additionally, phosphorylated eIF2 α induces translation of activating transcription factor 4 (ATF4), which triggers a transcriptional program of UPR^{ER} genes, including those associated with redox homeostasis and amino acid metabolism. ATF4 also upregulates the apoptosis-inducing CCAAT/Enhancer Binding Protein (C/EBP) homologous protein (*CHOP*) and the growth arrest and DNA damage-inducible gene 34 (*GADD34*)¹⁸⁴, which promotes dephosphorylation of eIF2 α to reverse translational inhibition^{185,186}.

Another ER stress transducer found in higher eukaryotes is ATF6 (ATF-6 in *C. elegans*), which is a basic leucine zipper protein (bZIP)-containing type II transmembrane transcription factor protein. ATF6 differs from IRE1 α and PERK in its domain architecture and mode of action¹⁸⁷. The luminal domain of ATF6 contains a Golgi apparatus localization sequence, which is shielded by its association with BiP, keeping it anchored to the ER membrane in the absence of ER stress. ATF6

becomes active after translocation to the Golgi apparatus, where it is proteolytically cleaved by site 1 and site 2 proteases (S1P and S2P)¹⁸⁸. This processing releases the cytosolic N-terminal bZIP transcription factor domain (ATF6n), which subsequently translocates to the nucleus and upregulates the expression of UPR^{ER} target genes, including *XBP1*, ER chaperones, and ERAD components^{182,187,189}.

Importantly, active ATF6n has synergistic and modulatory roles with the other two ER stress transduction branches IRE1 α and PERK. ATF6 transactivates *XBP1*, providing additional *XBP1* mRNAs that can be spliced by active IRE1 α ^{172,190}. Additionally, ATF6n can heterodimerize with XBP1s to enhance the expression of ERAD components, ER chaperones, and UPR^{ER} mediators, including BiP and XBP1 in mammals^{191,192}. The dimerization status and function of ATF6n may vary over the course of ER stress. ATF6n upregulates the transcription of ER chaperone genes as a homodimer and switches to forming ATF6n and XBP1s heterodimers later during ER stress to promote ERAD¹⁹³. In response to chronic ER stress, mammalian ATF6n modulates *XBP1* splicing and promotes the expression of the ATF4 target gene *CHOP*, indicating ATF6n may also function as a modulator of the IRE1 α and PERK UPR^{ER} branches¹⁹⁴.

Aging

Aging leads to a decline in proteostasis capacity, which can particularly affect post-mitotic cells such as neurons. Aging-related declines in proteostasis contribute to abnormal protein aggregation and downstream cellular degeneration. In neurodegenerative disease, age-dependent pathological protein accumulation drives synaptic impairment and neuron loss. Several studies have highlighted the importance of the UPR^{ER} in the aging brain. Under basal conditions, UPR^{ER} chaperones such as BiP are decreased in several brain regions of aged mice¹⁹⁵⁻¹⁹⁷. When these animals experience cellular stress, the UPR^{ER} fails to properly activate, leading to the upregulation of apoptotic pathway mediators¹⁹⁵⁻¹⁹⁷. The IRE1 α /XBP1 branch may

be particularly critical during aging. Dietary restriction extends lifespan in multiple organisms and requires IRE-1/XBP-1 activity in *C. elegans*¹⁹⁸. Interestingly, constitutive XBP-1s activation in neurons can extend lifespan in *C. elegans* via XBP-1s-mediated non-cell autonomous signaling events¹⁹⁹. Age strongly drives risk for many protein aggregate-deposition diseases of the CNS; UPR^{ER} dysfunction has been implicated in several of these diseases, including AD, PD, HD, and ALS^{200,201}.

Tau proteinopathies

Although age-associated failure of proteostasis mechanisms such as the UPR^{ER} may contribute to pathological protein accumulation in neurodegenerative diseases, the misfolding and aggregation of proteins associated with neurodegenerative diseases may also cause ER stress, leading to subsequent UPR^{ER} activation. Because post-mitotic neurons survive longer than other cell types without cell proliferation, excessive UPR^{ER} signaling can lead to or exacerbate neurodegenerative diseases^{202,203}. Conversely, glial cells are susceptible to ER stress due to their highly developed secretory systems²⁰⁴. The UPR^{ER} seems to play a role in neurodegenerative pathogenesis and has recently been proposed as a potential therapeutic target^{144,205-211}.

Experimental data on the relationship between pathological protein accumulation in tauopathies and activation of the UPR^{ER} in both *in vitro* and *in vivo* models have been difficult to interpret due to their variability and contradictory results²¹². In postmortem brain tissue of AD patients, BiP induction and phosphorylation of PERK have been observed²¹³ and others have demonstrated increased splicing of *XBP1* as well as induction of its downstream targets²¹⁴. Emphasizing the potential importance of UPR^{ER} activation in AD, polymorphisms in the *XBP1* promoter have been associated with development of the disease, indicating a role for the UPR^{ER} IRE1/XBP1 branch in AD onset²¹⁵. Specific to pathological tau species, studies have demonstrated an association of

UPR^{ER} activation markers with early tau pathology such as phosphorylated tau in AD²¹⁶ and primary tauopathy²¹⁷ postmortem brain tissue.

The timing of UPR^{ER} activation in tauopathies has suggested the response might initially be protective in the early stages of disease, but sustained activation may ultimately contribute to neuronal death^{213,218}. In support of a protective role, overexpression of XBP1s in transgenic *D. melanogaster* expressing human A β ₁₋₄₂ peptide in neurons reduced neurotoxicity²¹⁹. Additionally, XBP1 loss of function in transgenic *D. melanogaster* expressing human mutant tau protein exacerbated neuronal death²²⁰, and a genetic screen identified *xbp-1* as a modifier of tauopathy in a transgenic *C. elegans* model expressing human mutant tau protein²²¹.

Understanding how UPR^{ER} activation changes during the course of aging and how this contributes to the aging process may lead to important therapeutic interventions for aging-associated diseases such as neurodegeneration. However, the role of the UPR^{ER} in neurodegenerative diseases such as tauopathies has not been systematically studied. The work presented in this dissertation will address the role of the UPR^{ER} in *C. elegans* models of human wildtype tau pathology by characterizing: the involvement of the three UPR^{ER} branches (**Chapter II**); the involvement of the XBP-1s transcription factor and crosstalk between the other two UPR^{ER} branches (**Chapter II**); the involvement of XBP-1s transcriptional activation and its association with the ATF-6n transcription factor (**Chapter III**), and the potential mechanisms underlying transcriptional remodeling of the UPR^{ER}, which could inform translational studies in mammalian systems exploring therapeutic targets (**Chapter IV**).

Data

Table 1.1: Diseases with tau inclusions

Aging-related tau astrogliopathy
Alzheimer's disease
Amyotrophic lateral sclerosis/parkinsonism-dementia complex
Anti-IgLON5-related tauopathy
Argyrophilic grain disease
Chronic traumatic encephalopathy
Corticobasal degeneration
Diffuse neurofibrillary tangles with calcification
Down's syndrome
Familial British dementia
Familial Danish dementia
Frontotemporal dementia with parkinsonism linked to chromosome 17 (caused by <i>MAPT</i> mutations)
Frontotemporal lobar degeneration (some cases caused by <i>C9ORF72</i> mutations)
Gerstmann-Straussler-Scheinker disease
Globular glial tauopathies
Guadeloupean parkinsonism
Myotonic dystrophy
Neurodegeneration with brain iron accumulation
Niemann-Pick disease, type C
Non-Guamanian motor neuron disease with neurofibrillary tangles
Pantothenate kinase-associated neurodegeneration
Parkinsonism dementia complex of Guam
Primary age-related tauopathy
Picks's disease
Postencephalitic parkinsonism
Prion protein cerebral amyloid angiopathy
Progressive subcortical gliosis
Progressive supranuclear palsy
SLC9A6-related mental retardation
Subacute sclerosing panencephalitis
Tangle-only dementia
White matter tauopathy with globular glial inclusions

Table 1.2: Transgenic *C. elegans* models of human tauopathies

Promoter	Human Tau Isoform	Mutation	Phenotype	Reference
<i>rgef-1</i> (pan-neuronal)	0N3R	Wildtype	Mild (Uncoordinated locomotion)	222
		PHP sites (S198E, S199E, S202E, T231E, S235E, S396E, S404E, S409E, S413E)	Moderate (Uncoordinated locomotion, Insoluble tau, Neuronal abnormalities)	
		Non-PHP sites (S198A, S199A, S202A, T231A, S235A, S396A, S404A, S409A, S413A)	Moderate* (Uncoordinated locomotion, Neuronal abnormalities, Reduced lifespan)	
<i>mec-7</i> (mechanosensory neurons)	0N4R	Wildtype	Mild (Reduced touch response, Tau accumulation, Neuronal abnormalities)	223
		P301L	Severe (Reduced touch response, Tau accumulation, Neuronal abnormalities)	
		R406W	Most Severe (Reduced touch response, Tau accumulation, Neuronal abnormalities)	
<i>unc-119</i> (pan-neuronal)	0N3R	Wildtype	Uncoordinated locomotion, Neuronal abnormalities, Microtubular loss	224
	0N4R	Wildtype, P301L R406W		
<i>aex-3</i> (pan-neuronal)	1N4R	Wildtype	Moderate (Uncoordinated locomotion, Insoluble tau accumulation, Neuronal loss, Reduced lifespan)	130
		P301L	Severe (Uncoordinated locomotion, Insoluble tau accumulation, Neuronal loss, Reduced lifespan)	
		V337M [Tau (mutant)]	Most Severe (Uncoordinated locomotion, Insoluble tau accumulation, Neuronal loss, Reduced lifespan)	
<i>aex-3</i> (pan-neuronal)	1N4R	Wildtype [Tau (high)]	Severe (Uncoordinated locomotion, Insoluble tau, neuronal loss, reduced lifespan)	131,132
		Wildtype [Tau (low) A]	Mild (Uncoordinated locomotion, Neuronal abnormalities)	
<i>aex-3</i> (pan-neuronal)	1N4R	Wildtype [Tau (low) B]	Mild (Uncoordinated locomotion, Neurodegeneration)	132
<i>aex-3; rab-3</i> (pan-neuronal, double-transgenic)	1N4R; 4R fragment (aa 258-360)	V337M; Δ K280 (pro-aggregant)	Severe (Uncoordinated locomotion, Insoluble tau accumulation, Neuronal abnormalities)	225
		V337M; Δ K280, I277P, I308P (anti-aggregant)	Mild (Uncoordinated locomotion, Insoluble tau accumulation, Neuronal abnormalities)	

<i>aex-3</i> (pan-neuronal)	1N4R	Wildtype, A152E, A152T	Uncoordinated locomotion, Developmental toxicity, Impaired retrograde axonal transport, Neurodegeneration, Learning/memory deficits, Reduced lifespan	226
<i>ptl-1</i> (touch neurons)	2N4R	Wildtype	Reduced touch response, Neuronal abnormalities, Reduced lifespan	122
<i>snb-1</i> (pan-neuronal)	2N4R	Wildtype	Mild (Uncoordinated locomotion; dose-dependent)	227
		A152T	Severe (Uncoordinated locomotion, Neuronal loss, Reduced lifespan)	
	2N fragment (aa 1-243)	Wildtype, A152T	No obvious phenotype	
<i>aex-3</i> (pan-neuronal)	2N4R	Wildtype, V363A, V363I	Uncoordinated locomotion, Pharyngeal dysfunction, Insoluble tau accumulation, Synaptic impairment, Reduced lifespan	228

PHP: pseudohyperphosphorylated; *: higher expression level compared to other two transgenic models, which might cause phenotypes observed; aa: amino acid.

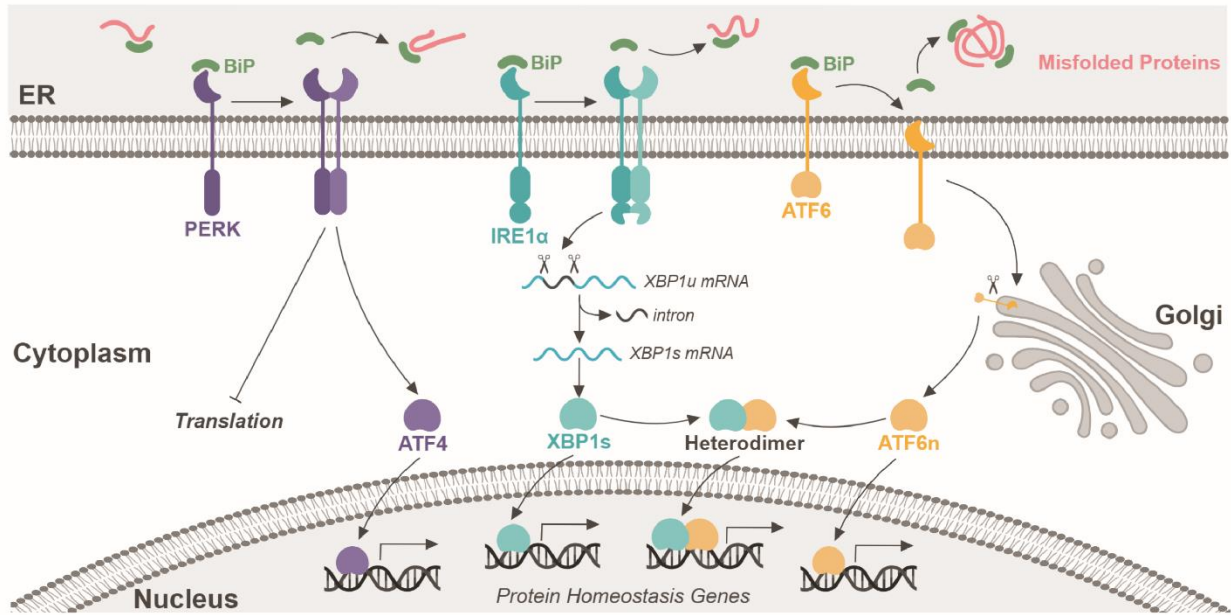


Figure 1.1: Simplified schematic of the mammalian UPR^{ER}.

CHAPTER II:

Constitutive XBP-1s-mediated activation of the endoplasmic reticulum unfolded protein response protects against pathological tau

Summary

The work in this chapter has been published (text and data obtained with permission from *Nature Communications*)¹³².

Introduction

Persisting for decades, human neurons face unique metabolic challenges, including the need to maintain long-term protein quality control (**Chapter I**). Cellular systems such as the cytoplasmic heat shock response and the unfolded protein responses in the ER (UPR^{ER}) and mitochondria (UPR^{mt}) enable neurons to maintain proteostasis within different cellular compartments. Proteostasis imbalance in the ER triggers the UPR^{ER}, which causes the molecular chaperone BiP to dissociate from the three ER transmembrane stress sensors PERK, IRE1 α , and ATF6 (**Figure 2.1a**). This results in immediate inhibition of general translation to decrease the protein folding load in the ER and activation of genes for restoring protein folding homeostasis. If the stress is prolonged and unresolved, the UPR^{ER} switches from a pro-survival role to a pro-apoptotic role, activating downstream signaling events of all three stress sensors to induce cell death. The UPR^{ER} is an evolutionarily conserved adaptive signaling pathway (**Chapter I**). The most conserved UPR^{ER} signaling branch, and the only one present in yeast, is initiated by IRE1 α ^{165,166}.

UPR^{ER} malfunctions are associated with a wide range of disease states, including tumor progression and diabetes, as well as immune, inflammatory, and neurodegenerative diseases²⁰¹. Although the UPR^{ER} plays many important physiological roles, we still do not fully understand the role of the UPR^{ER} in post-mitotic neuronal survival. Pathological tau protein serves as both a marker of brain aging and a hallmark of neurodegenerative diseases termed tauopathies (**Chapter I**). Tauopathies exhibit cytoplasmic accumulation of neuronal and/or glial lesions containing hyperphosphorylated tau. Interventions for tauopathies are limited to treatment of symptoms, as no approved therapies impact tau pathology or concomitant neurodegeneration.

To study tauopathies in a genetically tractable system, our laboratory has generated transgenic *C. elegans* that express human tau pan-neuronally (**Chapter I**)¹³⁰⁻¹³². The Tau (mutant) *C. elegans* model expresses FTDP-17 V337M mutant tau and exhibits behavioral abnormalities, accumulation of insoluble phosphorylated tau protein, progressive neurodegeneration, and shortened lifespan¹³⁰. Our laboratory previously conducted a genome-wide RNAi screen to identify modifiers of tau toxicity in Tau (mutant) *C. elegans*²²¹. From this screen, RNAi targeting the UPR^{ER} master transcription factor *xbp-1* enhanced tau-induced behavioral deficits, and strong *xbp-1* loss of function caused synthetic lethality with tau expression, nominating the UPR^{ER} as a regulator of tau proteostasis²²¹. The relationship between UPR^{ER} function and pathological tau accumulation remains poorly characterized (**Chapter I**). To study the consequences of UPR^{ER} loss of function on human wildtype tau toxicity in *C. elegans*, I utilized loss-of-function mutations and gain-of-function transgenes to genetically dissect the role of the UPR^{ER} in tau proteostasis.

Methods

C. elegans strains and transgenics

C. elegans strains used are listed in **Supplementary Table 2.1**. All strains were maintained at 20°C on standard nematode growth media plates containing OP50 *Escherichia coli* (*E. coli*)¹⁰⁶. *C. elegans* were grown on nematode growth media plates containing five times more peptone (5X PEP) prior to collection for protein and RNA extraction. The *C. elegans* husbandry and experimentation was conducted in accordance with all relevant ethical and safety regulations for animal testing and research.

C. elegans behavioral analysis

C. elegans swimming behavior was assessed as described previously¹³⁰. *C. elegans* were developmentally synchronized using a timed egg-lay and grown until all reached day one of adulthood at 20°C. Individual developmentally synchronized animals were placed in a 10 µL M9 buffer droplet on a 10 well Teflon-printed glass slide and allowed to acclimate to a liquid environment for 10 seconds. One thrash was defined as a bend across the midline or two consecutive bends from the midline toward the same side. The number of thrashes were counted during a one minute period and averaged for each strain. At least three biological replicate assay sessions with at least 10 animals per assay session were analyzed for statistical significance. I conducted behavioral analysis blinded to genotype to avoid biases. However, when the strains to be assayed were visibly distinguishable, blinding was not feasible. For comparison of two groups, an unpaired *t*-test, two-tailed, was used. For comparisons of three or more groups, a one-way analysis of variance (ANOVA), followed by Tukey's multiple comparison post-test was used.

C. elegans neurodegeneration assay

Analysis of *C. elegans* neurodegeneration was performed using a transgenic reporter strain with fluorescently marked VD and DD type inhibitory motor neurons¹³⁰. *C. elegans* were crossed with the EG1285 strain (*unc-47p::gfp*)²²⁹ to visualize the ventral nerve cord green fluorescent protein (GFP) marked gamma-aminobutyric acid (GABA)ergic neurons via fluorescence microscopy. *C. elegans* were developmentally synchronized using a timed egg-lay and grown until all reached day one or four of adulthood. *C. elegans* were mounted on glass slides with a 2% agarose gel pad and 10 μ L 0.1% sodium azide. The number of intact ventral nerve cord GABAergic D-type motor neurons were counted under fluorescence microscopy on a DeltaVision Elite (GE, Issaquah, WA, USA) imaging system using an Olympus 60x oil objective. Representative images of neuronal loss were also taken using an Olympus 60x oil objective. The three most rostral GABAergic motor neurons were excluded from counts due to occlusion from pharyngeal GFP co-injection markers. Therefore, the number of visibly accessible ventral nerve cord D-type motor neurons were counted out of a total of 16. Neurodegeneration was plotted as the average number of neurons lost from the 16 possible neurons analyzed. Two biological replicate assay sessions with at least 10 animals per assay session were analyzed for statistical significance. For comparison of two groups, an unpaired *t*-test, two-tailed, was used. Representative anatomical images were created with ImageJ Java²³⁰ to overlay images from the POL and GFP channels, with the GFP channel image re-colored green and the POL channel image re-colored grey for emphasis. Images from the GFP channel were also shown below merged images for visual clarity of neuronal loss.

C. elegans protein extraction

Protein was extracted from *C. elegans* using a rigorous lysis approach¹³⁰. *C. elegans* were synchronized using hypochlorite treatment and grown until adulthood at 20°C on 5X PEP plates.

Synchronized adult *C. elegans* were washed off 5X PEP plates in M9 buffer, washed an additional four times in M9 buffer to remove excess OP50 *E. coli*, pelleted by a one minute centrifugation at 800g, snap frozen in liquid nitrogen, and stored at -70°C until protein extraction. A total of two µL of high-salt reassembly (RAB) buffer (0.1 M 2-(N-morpholino)ethanesulfonic acid, 1 mM ethylene glycol bis-2-aminoethyl ether-N,N',N'',n'-tetraacetic acid, 0.5 mM MgSO₄, 0.75 M NaCl, 0.02 M NaF, pH 7.0) containing phenylmethylsulfonyl fluoride and protease inhibitors was added per milligram of packed worm pellet wet weight and homogenized via sonication four times at 70% power for eight seconds. This total soluble worm lysate was reserved for subsequent immunoblotting.

Protein immunoblotting

C. elegans protein immunoblotting was performed using Criterion apparatus (Bio-Rad) as recommended by the manufacturer (Bio-Rad Laboratories, Hercules, CA, USA)¹³⁰. *C. elegans* protein preparations were diluted 5:1 with sample buffer (0.046 M Tris, 0.005 M ethylenediamine tetraacetate, 0.2 M dithiothreitol, 50% sucrose, 5% sodium dodecyl sulfate, 0.05% bromophenol blue), boiled for 10 minutes, and centrifuged at 16,100g for two minutes. A total of 10 µL of diluted protein preparations were loaded and resolved on precast 4 to 15% gradient sodium dodecyl sulfate polyacrylamide (SDS-PAGE) gel and transferred to polyvinylidene difluoride (PVDF) membrane as recommended by the manufacturer (Bio-Rad Laboratories, Hercules, CA, USA). PVDF membranes were blocked in 5% milk in phosphate-buffered saline (PBS) for one hour before overnight incubation with primary antibody at 4°C. The next day, PVDF membranes were washed in PBS with 0.05% Tween, incubated at room temperature with horseradish peroxidase (HRP)-coupled secondary antibody for two hours, and washed in PBS with 0.05% Tween before visualization. The dilutions and pertinent details for all primary and secondary antibodies used are listed in Supplementary Table 2. Enhanced chemiluminescence substrate (Bio-Rad Laboratories, Hercules, CA, USA) was added to the PVDF membrane, and chemiluminescence signals were

detected with ChemiDoc-It®² 510 Imager (UVP LLC, Upland, CA, USA). Relative intensity of chemiluminescence signals was measured with ImageJ Java²³⁰. At least three biological assay replicates were analyzed for statistical significance. For comparison of two groups with biological replicates run on independent or single immunoblots, a paired *t*-test, two-tailed, was used. For comparisons of three or more groups run on independent or single immunoblots, a repeated measures ANOVA, followed by Tukey's multiple comparison post-test was used.

C. elegans freeze cracking for immunofluorescent staining

C. elegans immunofluorescent staining was performed according to standard protocols as described by Janet Duerr^{130,231}. Tau (high) animals were crossed with the CX10344 kyEx2454 strain (*unc-25::calf-1::gfp*, *unc-25::CP450::mCherry*, *odr-1::dsRED*)²³² to visualize the subcellular localization of tau protein relative to the endoplasmic reticulum via fluorescence microscopy. *C. elegans* were developmentally synchronized using a timed egg-lay and grown until all reached day one of adulthood at 20°C. Individual developmentally synchronized animals were placed in a 20 µL M9 buffer droplet on a 10 well Teflon-printed glass slide and washed to remove excess OP50 *E. coli* by moving to successive 20 µL M9 buffer droplets three times. Washed animals were transferred to a 10 µL 1% paraformaldehyde solution droplet on a poly-L-lysine coated glass slide and freeze cracked²³¹. Adherent animals were exposed to a light fixation using methanol-acetone for 5 minutes each at -20°C. Freeze cracked, fixed animals were stained and washed as described. Animals were counterstained with 300 nM DAPI and mounted with ProLong™ Gold antifade (Invitrogen, Carlsbad, CA, USA) before super-resolution microscopy analysis. The dilutions and pertinent details for all primary and secondary antibodies used are listed in **Supplementary Table 2.2**. Three-dimensional structured illumination microscopy (3D-SIM) images of animals were acquired on a Nikon N-SIM-E Ti2 microscope (Tokyo, Japan) using a CFI SR HP Apochromat TIRF 100XC Oil (NA 1.49) objective, 561/647 nm diode lasers, and an ORCA-Flash 4.0 sCMOS camera (Hamamatsu Photonics K.K., Naka-ku, Hamamatsu City, Shizuoka,

Japan). Image reconstruction and colocalization analysis was conducted using NIS-Elements (Nikon, Minato, Tokyo, Japan).

C. elegans RNA extraction

RNA was purified from snap-frozen packed *C. elegans* pellets using TRIzol Reagent as directed by the manufacturer's instructions²³³. RNA was resuspended in 50 uL sterile water. RNA concentration and purity were assessed using a NanoPhotometer® NP80 spectrophotometer (Implen GmbH, Munich, Germany). RNA integrity was assessed by 1% Tris/Borate/EDTA (TBE) agarose gel electrophoresis.

Quantitative RT-PCR

cDNA was prepared using iScript™ Reverse Transcription Supermix for RT-qPCR (Bio-Rad Laboratories, Inc., Hercules, CA, USA) as recommended by the manufacturer²³³. The primer set used to measure human *MAPT* gene was (forward primer: 5'-GTGTGGCTCATTAGGCAACATCC-3', reverse primer: 5'-CGTTCTCGCGGAAGGTCAG-3'). The primer set used for normalization was designed to detect *C. elegans* *rpl-32* gene (forward primer: 5'-GGTCGTCAAGAAGAAGCTCACCAA-3', reverse primer: 5'-TCTGCGGACACGGTTATCAATTCC-3'). Each genotype was tested with three biological replicates (including three technical replicates within each experiment) for each primer set. qPCR was performed using the iTaq™ Universal SYBR® Green Supermix kit (Bio-Rad Laboratories, Inc., Hercules, CA, USA) in a 384 well plate on a 7900HT Fast Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). Data were normalized within samples using an internal reference control gene (*rpl-32*).

Statistical analysis

Statistical significance for all phenotype assays was determined using GraphPad Prism statistical software (GraphPad Software, Inc., La Jolla, CA, USA). Statistical significance is demarcated in figures as *: $p < 0.05$, **: $p < 0.005$, and ***: $p < 0.0001$.

Schematic illustrations

Original schematic illustrations were created using an academic license with the online application BioRender (Toronto, ON, CA).

Results

UPR^{ER} branches differentially affect tauopathy phenotypes

The UPR^{ER} consists of three branches that regulate signaling to the nucleus and control the responses to unfolded protein within the ER lumen (**Figure 2.1a**). Using the genetically tractable model organism *C. elegans*, I examined the effects of loss-of-function mutations ablating signaling through each of the three branches of the UPR^{ER} [*pek-1* (-/-), *atf-6* (-/-), and *xbp-1* (-/-)] on tau-mediated behavioral dysfunction in a mild model of human wildtype tau toxicity, referred to as Tau (low) transgenic *C. elegans*¹³¹. Compared to non-transgenic (non-Tg) animals, Tau (low) animals exhibit mild behavioral deficits and no significant accumulation of pathological tau species.

C. elegans with a *pek-1* loss-of-function mutation [*pek-1* (-/-)] had normal locomotion (**Supplementary Figure 2.1a**). If *pek-1* regulates tau proteostasis, I would expect Tau (low); *pek-1* (-/-) animals to exhibit an enhancement of tau-dependent behavioral dysfunction. However, when crossed with Tau (low) animals, *pek-1* (-/-) did not worsen Tau (low) behavioral defects

(**Figure 2.1b**), suggesting the PEK-1 branch of the UPR^{ER} does not mediate tauopathy phenotypes in *C. elegans*.

C. elegans with an *atf-6* loss-of-function mutation [*atf-6* (-/-)] had normal locomotion behavior (**Supplementary Figure 2.1b**). However, Tau (low); *atf-6* (-/-) animals displayed worsened behavioral impairment compared to Tau (low) *C. elegans* (**Figure 2.1c**). To investigate whether *atf-6* loss of function in a Tau (low) background altered tau levels, I analyzed total tau protein expression by immunoblot. I found *atf-6* loss of function did not significantly affect total tau protein levels (**Figure 2.1d, e**). Therefore, *atf-6* loss of function enhances tau locomotion dysfunction independent from a role in tau protein turnover.

C. elegans with an *xbp-1* loss-of-function mutation [*xbp-1* (-/-)] had mild locomotion defects when compared to non-Tg animals (**Supplementary Figure 2.2**); however, Tau (low); *xbp-1* (-/-) *C. elegans* exhibited dramatically exacerbated tau-dependent locomotion dysfunction (**Supplementary Figure 2.3**). The mild behavioral deficits seen in Tau (low) animals were significantly enhanced with *xbp-1* loss of function. This phenotype was confirmed using an independent tau transgenic *C. elegans* (**Figure 2.2a**). To determine the effects of *xbp-1* loss of function on tau protein turnover, I assayed tau protein levels by immunoblot. Tau (low); *xbp-1* (-/-) animals exhibited increased tau protein compared to Tau (low) animals (**Figure 2.2b, c**).

Neurodegeneration is the ultimate consequence of pathological tau in tauopathy disorders. To assess whether *xbp-1* loss of function promotes tau-dependent neurodegeneration, I used a transgenic *C. elegans* reporter strain expressing green fluorescent protein (GFP) in D-type gamma-aminobutyric acid (GABA) positive neurons (*unc-47p::gfp*)²²⁹. This strain allows *in vivo* assessment of the integrity of GABAergic motor neurons along the ventral nerve cord. At day 1 of adulthood, Tau (low) animals did not exhibit significant neurodegeneration (**Figure 2.2d, e**).

Fewer than one in four Tau (low) animals were missing one neuron (**Figure 2.2e**). However, at day 1 of adulthood, *xbp-1* (-/-) exacerbated neurodegeneration in Tau (low) animals, including loss of neuronal cell bodies and breaks in the ventral nerve cord (**Figure 2.2d**). Nearly every Tau (low); *xbp-1* (-/-) animal exhibited neurodegeneration (**Figure 2.2e**). Loss of GABAergic motor neurons was more pronounced at day 4 of adulthood (**Figure 2.2f**). Importantly, *xbp-1* (-/-) animals did not exhibit neurodegeneration on their own in the absence of tau (**Supplementary Figure 2.4**). Taken together, *xbp-1* loss of function exacerbates tau-mediated behavioral deficits, increases accumulation of tau protein, and drives tau-dependent neurodegeneration in *C. elegans* models of tauopathy.

Neuronal xbp-1s activation ameliorates tauopathy phenotypes

Given *xbp-1* loss of function enhances tauopathy phenotypes, I hypothesized *xbp-1* gain of function might suppress tauopathy. To address this question, I utilized a severe model of tau toxicity, Tau (high) transgenic *C. elegans*¹³¹, which exhibits strong behavioral and neurodegenerative phenotypes driven by pathological tau protein¹³¹, enabling me to readily detect potential improvement in tauopathy phenotypes. I crossed Tau (high) transgenic animals with transgenic animals expressing a pan-neuronal constitutively active *xbp-1s* gain-of-function transgene (*xbp-1s* Tg), which promotes activation of the UPR^{ER} in the absence of increased ER protein folding load¹⁹⁹. Compared to non-transgenic (non-Tg) animals, Tau (high) animals exhibit major locomotion dysfunction and pronounced accumulation of pathological tau species¹³¹.

C. elegans expressing *xbp-1s* Tg have mild locomotion defects when compared to non-Tg animals (**Supplementary Figure 2.5**), while Tau (high) *C. elegans* have severe locomotion dysfunction (**Figure 2.3a**). However, Tau (high); *xbp-1s* Tg animals had robust rescue of the tau-mediated behavioral deficits compared to Tau (high) animals alone (**Figure 2.3a**). To test whether *xbp-1s* Tg promotes tau protein turnover, I examined tau levels by immunoblot. Tau (high); *xbp-*

1s Tg animals accumulated less total tau protein when compared to Tau (high) animals (**Figure 2.3b, c**). To address the possibility that *xbp-1s* hyperactivity decreases tau protein levels by an effect on tau transgene expression rather than protein turnover, I assessed human tau mRNA levels by quantitative reverse-transcription PCR (qRT-PCR). I found no significant difference in tau-encoding mRNA (**Supplementary Figure 2.6**), indicating reduced levels of tau protein are a result of increased tau protein clearance rather than decreased transgenic tau mRNA expression. In tauopathy disorders, tau becomes hyperphosphorylated, driving pathological protein aggregation and neurodegeneration²³⁴. I measured tau phosphorylation by immunoblot at four sites commonly associated with tauopathy (Threonine 181, Serine 202, Serine 396/404, and Serine 422) using phosphorylated tau-specific antibodies. Tau (high); *xbp-1s* Tg *C. elegans* had less phosphorylated tau at all four epitopes compared to Tau (high) animals (**Figure 2.3d, Supplementary Table 2.3**). I then tested whether *xbp-1s* gain of function would protect against tau-driven neurodegeneration. At day 1 of adulthood, approximately 50% of Tau (high) *C. elegans* lost one GABAergic neuron (**Figure 2.3e, f**). However, Tau (high); *xbp-1s* Tg worms were protected against this early tau-induced neuronal loss (**Figure 2.3e, f**). By day 4 of adulthood, Tau (high) worms showed progressive neuronal loss with an average of almost three neurons lost per animal, while Tau (high); *xbp-1s* Tg worms that exhibited neurodegeneration lost approximately one neuron (**Figure 2.3g**). Altogether, I have demonstrated *xbp-1s* gain of function can significantly suppresses behavioral, biochemical, and neurodegenerative tauopathy phenotypes observed in the *C. elegans* Tau (high) background.

ATF-6 mediates xbp-1s suppression of tauopathy

The XBP-1s transcription factor mediates one of three branches of the UPR^{ER} (**Figure 2.1a**). To understand whether the other two UPR^{ER} branches participate in *xbp-1s*-mediated tauopathy suppression in *C. elegans*, I again utilized *pek-1* and *atf-6* loss-of-function mutant strains. Tau (high); *xbp-1s* Tg animals were crossed with either *pek-1* or *atf-6* loss-of-function mutant strains.

I evaluated the consequences of deleting *pek-1* in Tau (high) animals alone and observed no change in tau-induced behavioral dysfunction (**Figure 2.4a**), consistent with my data for Tau (low) animals (**Figure 2.1b**). Next, I tested whether *pek-1* (-/-) could prevent *xbp-1s* suppression of tau toxicity. I found *pek-1* loss of function partially blocked the suppression of tau motility dysfunction in Tau (high); *xbp-1s* Tg; *pek-1* (-/-) animals (**Figure 2.4a**). I then examined the effect of *atf-6* (-/-) on Tau (high) animals. In contrast to my previous finding in Tau (low) animals (**Figure 2.1c**), I found Tau (high); *atf-6* (-/-) *C. elegans* had similar behavioral abnormalities when compared to Tau (high) animals (**Figure 2.4b**). The severity of behavioral dysfunction of the Tau (high) animals likely explains my inability to discriminate enhancement of the tauopathy phenotype. Finally, I tested whether *atf-6* (-/-) prevents *xbp-1s*-mediated suppression of tau toxicity. Surprisingly, I found *atf-6* loss of function completely blocked *xbp-1s*-mediated tauopathy suppression in Tau (high); *xbp-1s* Tg; *atf-6* (-/-) animals (**Figure 2.4b**). I confirmed these results with an independent *atf-6* loss-of-function allele (**Supplementary Figure 2.7a**).

Because *xbp-1s*-mediated tauopathy behavioral suppression requires *atf-6*, I also measured *atf-6* loss of function effects on total tau protein by immunoblot in Tau (high) animals using two independent *atf-6* loss-of-function alleles. Loss of function of *atf-6* alone did not change tau protein levels in Tau (high) animals (**Figure 2.4c, d**; **Supplementary Figure 2.7b**). I have shown *xbp-1s* gain of function reduced tau accumulation in Tau (high) animals (**Figure 2.3b, c**; **Figure 2.4c, d**; **Supplementary Figure 2.7b**). In contrast, Tau (high); *xbp-1s* Tg; *atf-6* (-/-) animals accumulated total tau protein similar to the level seen in Tau (high) animals (**Figure 2.4c, d**; **Supplementary Figure 2.7b**), indicating functional ATF-6 is necessary for *xbp-1s*-mediated tauopathy detoxification. Taken together, these data demonstrate distinct, but overlapping roles for the three branches of the UPR^{ER} in regulating tau proteostasis in transgenic *C. elegans*.

ERAD is required for xbp-1s-mediated tauopathy suppression

Dysregulated proteostasis within the ER triggers the UPR^{ER}, resulting in activation of ER-associated protein degradation (ERAD). This provides a means for the direct removal of misfolded proteins inside the ER lumen and facilitates their degradation through either autophagy or the proteosome²³⁵. To determine whether tau protein resides within the ER, I crossed Tau (high) animals with transgenic animals expressing a fluorescently-tagged ER integral membrane protein, CP450::mCherry²³². I co-immunostained transgenic animals with antibodies targeting tau and mCherry to visualize subcellular localization of these proteins (**Figure 2.5a-c**). Using co-localization immunofluorescence analysis, I showed no significant overlap of tau protein and the ER protein CP450 (**Figure 2.5d**). Thus, the mechanism of tau detoxification by UPR^{ER} activation in transgenic *C. elegans* does not rely upon removal of tau protein directly from the ER.

Previous work in cultured human cells demonstrated tau impairs ERAD function²³⁶. I hypothesized abnormal tau also inhibits ERAD in *C. elegans*, and neuronal tau toxicity in *C. elegans* may depend upon ERAD impairment. In support of this hypothesis, previous work has shown UPR^{ER} activation stimulates ERAD through XBP-1s target genes²³⁷. Therefore, XBP-1s suppression of tauopathy phenotypes in *C. elegans* may be through an ERAD-dependent mechanism. To test the involvement of ERAD in tau clearance, I examined whether ERAD loss of function could block XBP-1s suppression of tauopathy in *C. elegans*. SEL-11, the *C. elegans* homolog of the ERAD-associated E3 ubiquitin ligase HRD1, is required for ER-associated protein degradation²³⁸. I crossed *sel-11* loss-of-function mutants [*sel-11* (-/-)] with Tau (high); *xbp-1s* Tg animals and measured behavioral function. I found *sel-11* (-/-) fully blocked XBP-1s-mediated suppression of tauopathy behavioral deficits (**Figure 2.5e**). Taken together, these data suggest ERAD activation via XBP-1s promotes clearance of pathological tau in *C. elegans*.

Discussion

Although aging both increases AD risk and decreases UPR^{ER} function, the specific contribution of UPR^{ER} activity to disease initiation and/or progression remains poorly understood. Likewise, whether UPR^{ER} induction causes or results from AD pathology remains unclear. Most studies to date have made correlative associations or relied upon immortalized cell culture experiments, which fail to model key aspects of tauopathy²³⁹. Here, I have taken a more direct approach by genetically dissecting the role of the UPR^{ER} in tauopathy *in vivo* using human wild type tau transgenic *C. elegans*. I found *xbp-1s* expression to be a central control point in tauopathy, although this role requires involvement of all three branches of the UPR^{ER}.

To study the contributions of each UPR^{ER} branch, I systematically eliminated the function of the three UPR^{ER} branches in tau transgenic *C. elegans*. The PERK branch of the UPR^{ER} mainly functions to attenuate protein synthesis in the ER. Interestingly, I found *C. elegans pek-1* does not regulate tau proteostasis on its own (**Figure 2.1b**), indicating a non-essential role for this branch in this tauopathy model. However, the PERK branch of the UPR^{ER} plays a modest role in modulating tauopathy risk, as human *PERK* variants increase risk for developing PSP by approximately 25%²⁴⁰. In addition, human postmortem brain analyses found increased PERK activation in a subset of PSP²⁴¹ and AD disease-affected brain regions^{213,216,241}. My results indicate either the consequences of PEK-1 modulation are too modest to be detected in tau transgenic *C. elegans*, or there might be a critical species-specific difference between humans and *C. elegans* regarding PERK/PEK-1 function in the UPR^{ER}.

The XBP1 and ATF6 branches of the UPR^{ER} mediate transcriptional regulation of genes for restoring proteostasis in the ER. My results show both *atf-6* and *xbp-1* loss of function enhance tauopathy-induced behavior defects in *C. elegans* (**Figure 2.1c**, **Figure 2.2a**; **Supplementary Figure 2.3**), with *xbp-1* loss of function additionally increasing tau protein levels and

neurodegeneration (**Figure 2.2b-f**). In humans, a polymorphism in the promoter of the *XBP1* gene was found to be a risk factor for AD in a Chinese Han population²¹⁵. Postmortem tissue analyses have revealed a more complex scenario for XBP1 branch changes in AD, possibly related to disease stage, patient cohort characteristics, or experimental methodology. One group found the constitutively active form XBP1s is downregulated in AD brains²⁴². Another group found increased *XBP1s*, but no increase in its key regulatory target BiP, indicating a disturbance in UPR^{ER} activation²¹⁴. Work from our laboratory and others has demonstrated loss of UPR^{ER} function via XBP1 exacerbates pathological tau phenotypes in simple model organisms such as *C. elegans* (²²¹, this study¹³²) and *D. melanogaster*²²⁰.

Several recent studies highlight the potential for the UPR^{ER}, specifically XBP1, as a therapeutic target for dementia. First, upregulation of XBP1s rescues A β -mediated neurotoxicity in *D. melanogaster*, mammalian cultured neurons, and *C. elegans*^{219,243}. Second, *Xbp1* gain of function in the hippocampus of mice can improve spatial memory²⁴⁴. To dissect the role of UPR^{ER} induction in protection from tauopathy, I expressed constitutively active *xbp-1s* in *C. elegans* neurons. I found XBP-1s protects neurons from pathological tau by promoting proteostasis and clearance of abnormal tau species, resulting in amelioration of both behavioral dysfunction and neurodegenerative changes in *C. elegans* (**Figure 2.3**). Taken together, these data clearly support a critical role for UPR^{ER} transcriptional induction in mediating the clearance of abnormal protein species such as A β and tau, while shedding light on a novel active tau clearance mechanism in AD and other tauopathies.

Given the highly orchestrated nature of the UPR^{ER} (**Figure 2.1a**), I next investigated whether the other two branches of the UPR^{ER} were genetically required for XBP-1s-mediated protection against pathological tau in *C. elegans*. Interestingly, this experiment identified interplay between the three branches of the UPR^{ER}. I observed *C. elegans* PEK-1 loss of function limits but does

not eliminate *xbp-1s*-mediated suppression of tauopathy-induced behavioral deficits (**Figure 2.4a**). In *C. elegans*, the *ire-1/xbp-1* and *pek-1* branches play partially complementary roles in eliminating ER stress, where *ire-1/xbp-1* signals to activate UPR^{ER} transcription and *pek-1* signals to attenuate protein synthesis²⁴⁵. In addition, *pek-1* appears to be dispensable for the induction of genes directly involved in ER protein folding and ERAD¹⁸⁰. The limited role of *pek-1* in *xbp-1s*-mediated tauopathy suppression indicates a fully functioning UPR^{ER} is required to completely overcome cytoplasmic pathological tau protein accumulation in *C. elegans*. Nonetheless, transgenic overexpression of XBP-1s can partially suppress tauopathy even in the absence of *pek-1* activity.

In contrast, I have shown *atf-6* loss of function completely abolished *xbp-1s*-mediated tauopathy suppression in *C. elegans* (**Figure 2.4b-d; Supplementary Figure 2.7**). This demonstrates an absolute requirement for ATF-6 in mediating tauopathy suppression by transgenic constitutively active XBP-1s expression in *C. elegans* and a previously unappreciated critical crosstalk between ATF-6 and XBP-1 branches of the UPR^{ER} in tau proteostasis. Given the known importance of the XBP-1 and ATF-6 branches in UPR^{ER} transcriptional regulation, a plausible mechanism of tauopathy suppression involves regulation of specific target gene(s) coordinately upregulated by ATF-6n and XBP-1s transcription factors. In agreement with my results, previous studies suggest ATF-6n might have evolved as a backup mechanism to the XBP-1s transcriptional pathway in *C. elegans*, since many genes regulated by ATF-6n overlap with those regulated by XBP-1s¹⁸⁰. In mammals, ATF6 activates the transcription of *XBP1* and can form heterodimers with XBP1s to control the induction of specific patterns of gene expression^{191,192}. Additionally, mouse XBP1s/ATF6n heterodimers exhibit a higher binding affinity to target genes than XBP1s homodimers, indicating the strong influence of ATF-6n on XBP-1s transcriptional regulation¹⁹¹. Currently, it is unknown whether *C. elegans* XBP-1s and ATF-6n transcription factors can form heterodimers, but they do exhibit a high degree of sequence and functional conservation with

XBP1 and ATF6 homologs in mammals. Taken together with the requirement for ATF-6 function in XBP-1s-mediated tauopathy suppression, I hypothesize these two transcription factors also form heterodimers in *C. elegans* to promote tauopathy suppression. Alternatively, other scenarios involving an ordered pathway, where *atf-6* acts downstream of *xbp-1*, could explain the dependence of *xbp-1s* suppression on *atf-6*. Similarly, *atf-6* loss of function could cause synergistic deficits with pathological tau that *xbp-1s* overexpression cannot ameliorate. Regardless, the parallel findings for *atf-6* and *pek-1* loss of function suggests full UPR^{ER} functionality promotes *xbp-1s*-mediated tauopathy suppression in *C. elegans*.

I found upregulating the UPR^{ER} master transcription factor XBP-1s can increase tau protein turnover in the cytoplasm. Cytoplasmic tau can be degraded by at least two mechanisms: the UPS and autophagy. Soluble monomeric tau species are recognized by molecular chaperones and directed to the 20S proteasome without ubiquitination or 26S proteasome with ubiquitination for degradation, while aggregation-prone tau species, such as hyperphosphorylated tau, are degraded via autophagy^{144,246}. Both of these protein degradation mechanisms are utilized by the UPR^{ER} via ERAD, which shuttles proteins from the ER to the cytoplasm for degradation. Although tau does not reside in the ER, ERAD loss of function via *sel-11* (-/-) is essential for *xbp-1s*-mediated behavioral tauopathy suppression in *C. elegans* (**Figure 2.5**), highlighting the importance of trans-compartmental trafficking mechanisms for global proteostasis.

I propose the following mechanism explaining the relationship between cytoplasmic tau and the UPR^{ER} in neurons (**Figure 2.6**). Although tau is not an ER resident protein, abnormal tau in the cytoplasm can nevertheless trigger the UPR^{ER} by altering overall neuronal proteostasis through impaired ribosome function²⁴⁷, cytoplasmic/ER protein translocation, and disruption of ERAD²³⁶, leading to misfolded proteins in the ER. The main goal of the UPR^{ER} is to restore proteostasis within the ER lumen, but to achieve this requires integrated stress relief within both cytoplasm

and ER compartments. This is highlighted by a recent study demonstrating soluble tau impairs ERAD, which results in increased ER protein folding load and activation of the UPR^{ER} ²³⁶ (**Figure 2.6a**). Nascent proteins in the ER failing to properly fold are degraded through ERAD, a branch of the UPS in which proteins are ubiquitinated at the ER membrane for subsequent degradation. Although this is an ER-associated degradation pathway, the ERAD machinery can degrade abnormal proteins located outside the ER, such as in the cytoplasm or nucleus²⁴⁸. XBP-1s UPR^{ER} branch loss of function inhibits ERAD, leading to pathological accumulation of abnormal tau as it is converted from soluble normal tau in the cytoplasm (**Figure 2.6b**). In the presence of pathological cytoplasmic tau, XBP-1s UPR^{ER} branch gain of function stimulates high levels of ERAD, leading to turnover of pathological tau species, such as hyperphosphorylated tau (**Figure 2.6b**). Taken together with my findings, these studies indicate the importance of understanding the role of abnormal tau protein and the UPR^{ER} in age-associated neurodegenerative disease.

Future translational studies will need to address whether UPR^{ER} activation can protect against pathological tau in the mammalian brain. More broadly, exploring how different cellular compartments communicate to achieve neuronal proteostasis remains an important mechanistic question with therapeutic implications. Specifically, the mechanism of how cytoplasmic pathological tau feeds back on ER folding load needs to be identified. Given the importance of the two transcriptional branches of the UPR^{ER} in tauopathy, next I will identify *xbp-1s* target genes driving tau proteostasis in *C. elegans*, which could help inform more specific neuroprotective strategies in mammals. Likewise, involvement of the UPR^{ER} in tauopathy needs to be explored in the mammalian brain to clarify whether targeting the UPR^{ER} in tauopathy could have neuroprotective benefit.

Data

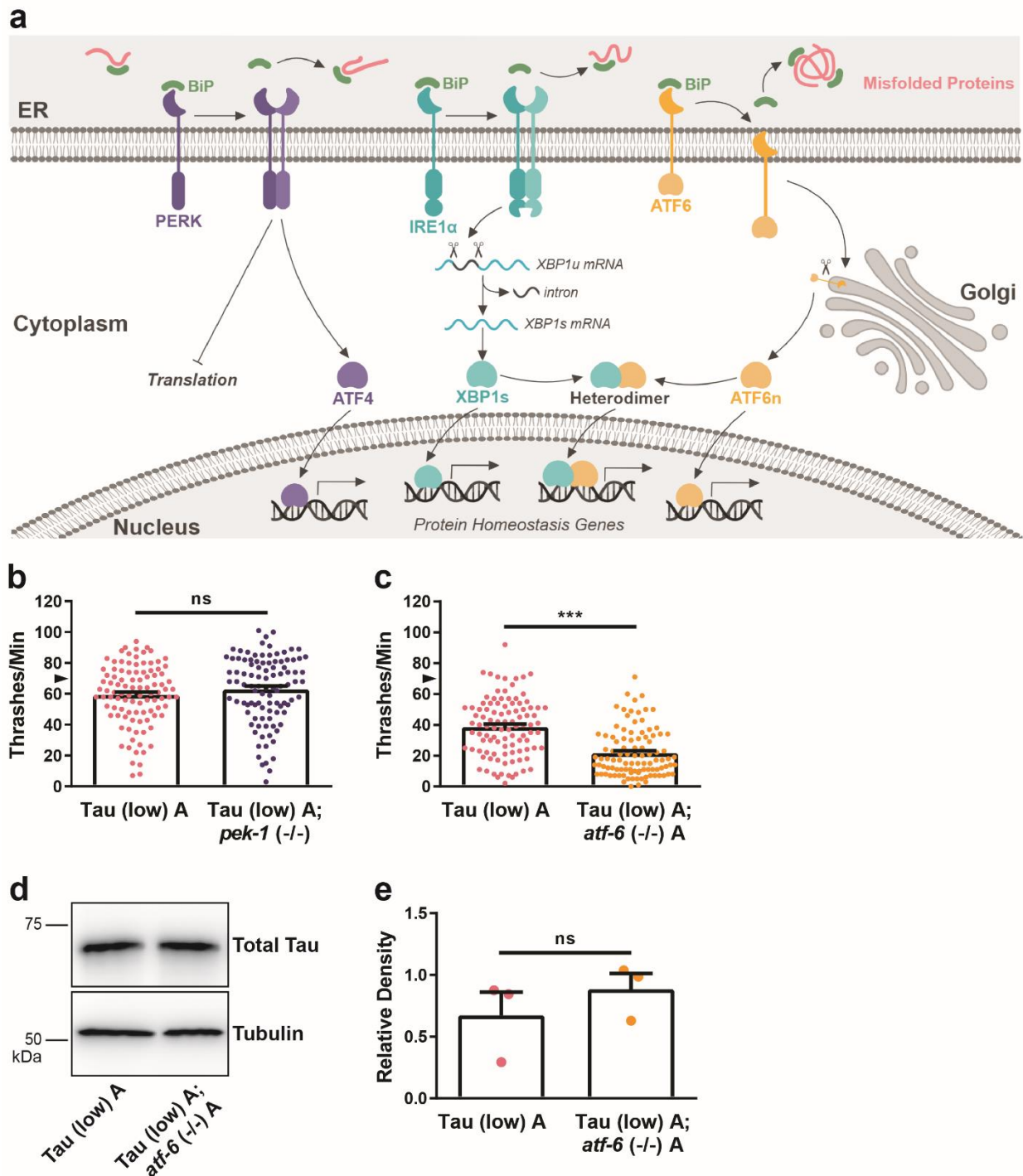


Figure 2.1: The UPR^{ER} PEK-1/PERK and ATF-6/ATF6 branches are differentially involved in tauopathy in transgenic *C. elegans*. **a** Model figure of the mammalian UPR^{ER}, which acts to restore ER proteostasis. Cellular stress can disrupt ER proteostasis, triggering the UPR^{ER} stress response. Three transmembrane stress sensors (PERK, IRE1 α , and ATF6) are bound by the ER molecular chaperone BiP, holding them in inactive conformations. Misfolded proteins in the ER

causes dissociation of BiP from the stress sensors, allowing adoption of active conformations. PERK dimerization and autophosphorylation allows phosphorylation of eIF2 α and other substrates in the cytoplasm. Active PERK attenuates overall protein translation to reduce nascent protein folding load in the ER and activates transcription factor ATF4 to control target genes involved in restoring proteostasis. IRE1 α dimerization promotes non-canonical splicing of *XBP1* mRNA in the cytoplasm to produce *XBP1s* mRNA encoding the UPR^{ER} master transcription factor XBP1s. ATF6 translocates to the Golgi apparatus for cleavage into N- and C-terminal fragments, with the N-terminal fragment becoming an active transcription factor (ATF6n). Additionally, XBP1s and ATF6n can form heterodimers to regulate transcriptional target genes essential for ER proteostasis restoration. **b** *pek-1* loss of function in Tau (low) animals does not alter mild behavioral defects observed in a liquid environment [$n = 100$ animals; $N = 5$ biologically independent experiments; statistical analysis is by unpaired *t*-test, two tailed (ns: $p = 0.2261$)]. **c** *atf-6* loss of function in Tau (low) animals enhances mild behavioral defects observed in a liquid environment [$n = 100$ animals; $N = 5$ biologically independent experiments; statistical analysis is by *t*-test, two tailed (***: $p < 0.0001$)]. **d, e** *atf-6* loss of function in Tau (low) animals does not affect soluble tau protein levels. Representative immunoblots for total tau and tubulin are shown, and densitometry analysis of chemiluminescence signals for tau normalized to tubulin are plotted [$N = 3$ biologically independent experiments; statistical analysis is by paired *t*-test, two-tailed (ns: $p = 0.4041$)]. All bar graphs represent mean + SEM. Arrowhead on y-axis of all liquid thrashing bar graphs denotes non-Tg animals average ~70 thrashes/minute under standard laboratory conditions.

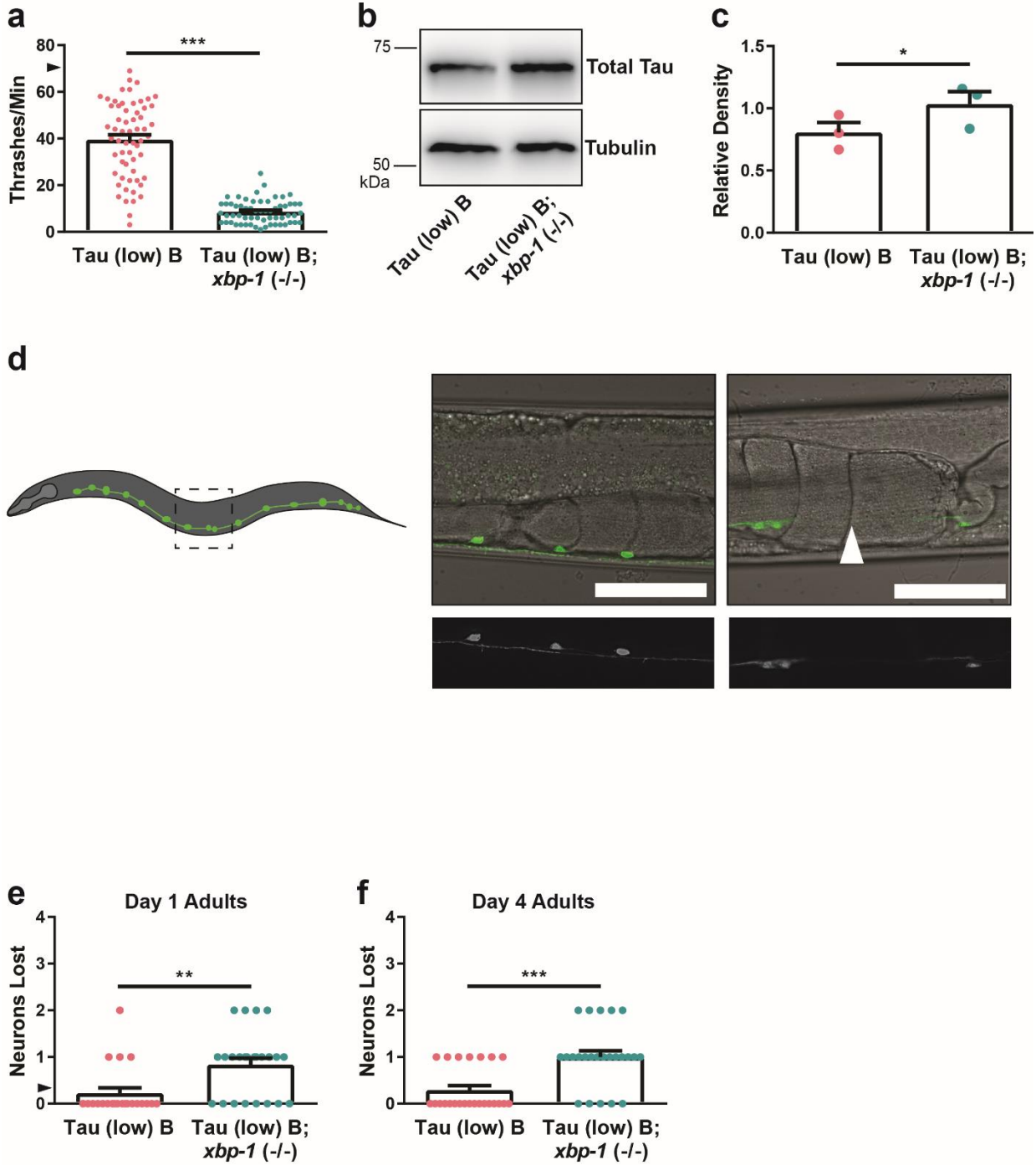


Figure 2.2: The UPR^{ER} XBP-1 branch loss of function enhances tauopathy in transgenic *C. elegans*. **a** *xbp-1* loss of function in Tau (low) animals enhances mild behavioral defects observed in a liquid environment [$n = 60$ animals; $N = 4$ biologically independent experiments; statistical analysis is by unpaired t -test, two tailed (***: $p < 0.0001$)]. **b, c** *xbp-1* loss of function in Tau (low) animals increases soluble tau protein levels. Representative immunoblots for total tau and tubulin are shown, and densitometry analysis of chemiluminescence signals for tau normalized to tubulin are plotted [$N = 3$ biologically independent experiments; statistical analysis is by paired t -test, two-tailed (*: $p = 0.0314$)]. **d-f** *xbp-1* loss of function in Tau (low) animals increases neuronal loss with

age. **d** Representative images of D-type GABAergic ventral nerve cord neurons at day one of adulthood. The typical Tau (low) animal did not exhibit neuron loss, while the Tau (low); *xbp-1* (-/-) animal exhibited loss of one neuron (white arrowhead = neuron loss; scale bar = 50 μ m). **e, f** The number of D-type GABAergic ventral nerve cord neurons lost at day one of adulthood [n = 22 and 24 animals, respectively; N = 2 biologically independent experiments; statistical analysis is by unpaired t -test, two-tailed (**: p = 0.0020] and day four of adulthood [n = 24 animals; N = 2 biologically independent experiments; statistical analysis is by unpaired t -test, two-tailed (***: p < 0.0001)] is plotted. All bar graphs represent mean + SEM. Arrowhead on y -axis of all liquid thrashing bar graphs denotes non-Tg animals average ~70 thrashes/minute under standard laboratory conditions.

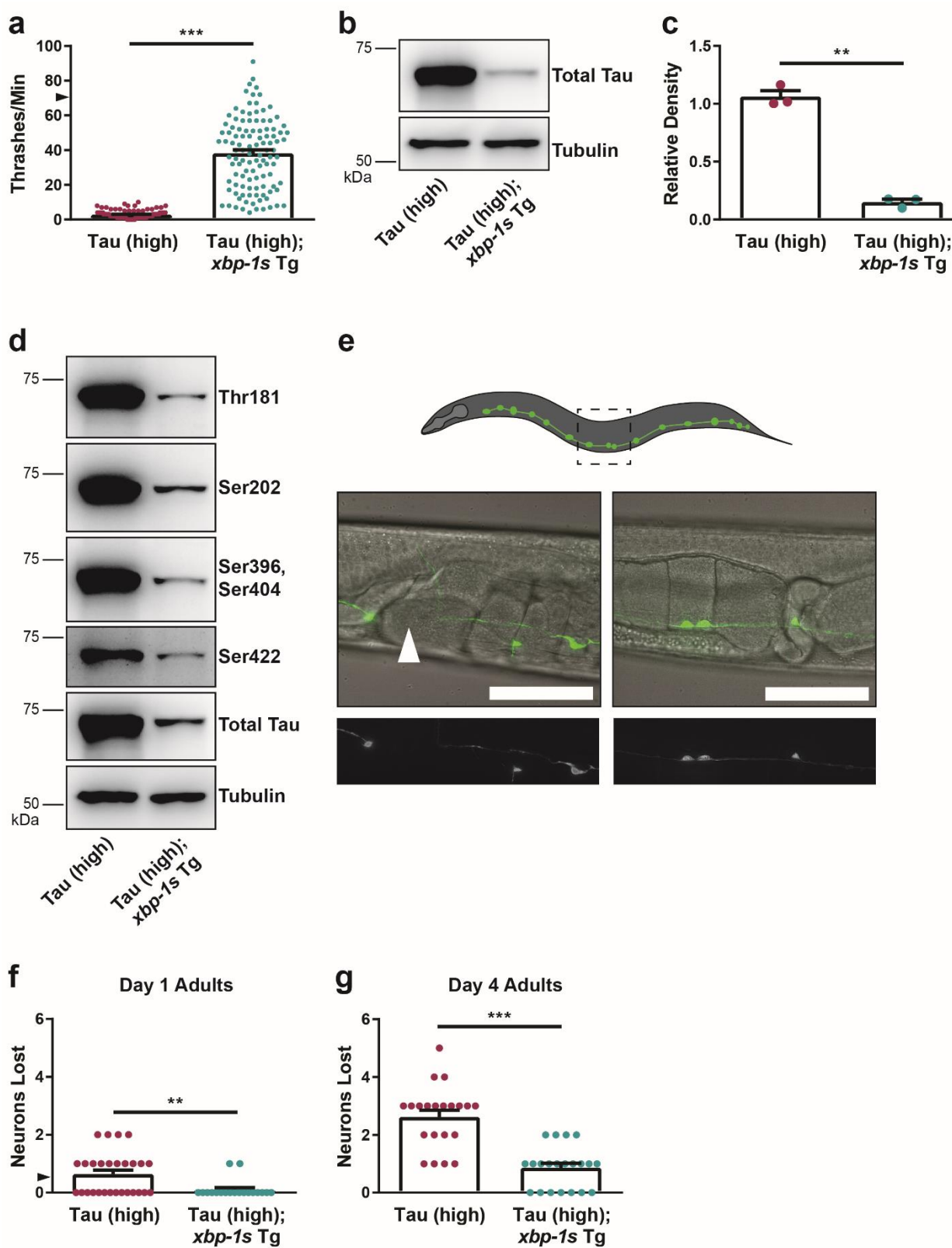


Figure 2.3: Constitutive UPR^{ER} activation by neuronal overexpression of *xbp-1s* suppresses tauopathy in transgenic *C. elegans*. a Neuronal overexpression of *xbp-1s* in Tau

(high) animals suppresses severe behavioral defects observed in a liquid environment [$n = 110$ animals; $N = 6$ biologically independent experiments; statistical analysis is by unpaired t -test, two-tailed (***: $p < 0.0001$)]. **b-d** Neuronal overexpression of *xbp-1s* in Tau (high) animals decreases different soluble tau protein species. **b, c** Representative immunoblots for total tau and tubulin are shown, and densitometry analysis of chemiluminescence signals for total tau normalized to tubulin are plotted [$N = 3$ biologically independent experiments; statistical analysis is by paired t -test, two-tailed **: $p = 0.0022$]. **d** Representative immunoblots for phosphorylated tau (Threonine 181, Serine 202, Serine 396/Serine 404, Serine 422 phosphorylation sites), total tau, and tubulin are shown. Densitometry analysis of chemiluminescence signals for phosphorylated tau species normalized to total tau and tubulin are provided in Supplementary Table 2.3 ($N = 4$ biologically independent experiments; statistical analysis is by paired t -test, two-tailed). **e-g** Neuronal overexpression of *xbp-1s* in Tau (high) animals decreases neuronal loss with age. **e** Representative images of D-type GABAergic ventral nerve cord neurons at day one of adulthood. The typical Tau (high) animal exhibited loss of one neuron, while the Tau (high); *xbp-1s* (Tg) animal did not exhibit neuron loss (white arrowhead = neuron loss; scale bar = 50 μm). **f, g** The number of D-type GABAergic ventral nerve cord neurons lost at day one of adulthood [$n = 28$ and 19 animals, respectively; $N = 2$ biologically independent experiments; statistical analysis is by unpaired t -test, two-tailed (**: $p = 0.0042$)] and day four of adulthood [$n = 21$ animals; $N = 2$ biologically independent experiments; statistical analysis is by unpaired t -test, two-tailed (***: $p < 0.0001$)] is plotted. All bar graphs represent mean + SEM. Arrowhead on y-axis of all liquid thrashing bar graphs denotes non-Tg animals average ~70 thrashes/minute under standard laboratory conditions.

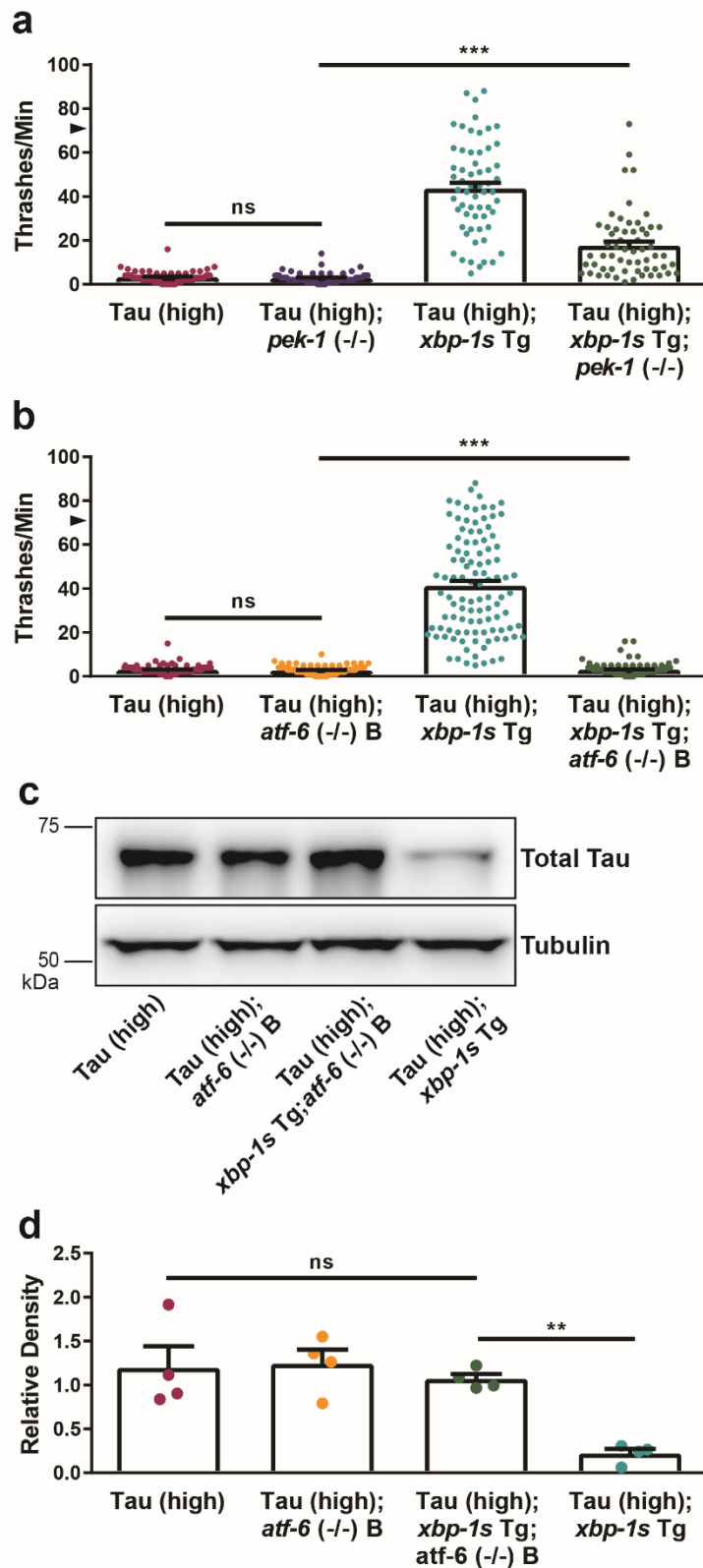


Figure 2.4: The UPR^{ER} branches are differentially involved in *xbp-1s*-mediated tauopathy suppression in transgenic *C. elegans*. **a** *pek-1* loss of function partially blocks the ability of

neuronal overexpression of *xbp-1s* in Tau (high) animals to suppress severe behavioral defects observed in a liquid environment [$n = 60$ animals; $N = 4$ biologically independent experiments; statistical analysis is by one-way ANOVA, followed by Tukey's post-test (***: $p < 0.0001$)]. **b** *atf-6* loss of function abolishes the ability of neuronal overexpression of *xbp-1s* in Tau (high) animals to suppress severe behavioral defects observed in a liquid environment [$n = 105$ animals; $N = 7$ biologically independent experiments; statistical analysis is by one-way ANOVA, followed by Tukey's post-test (***: $p < 0.0001$)]. **c, d** *atf-6* loss of function abolishes the ability of neuronal overexpression of *xbp-1s* in Tau (high) animals to decrease soluble tau protein levels. Representative immunoblots for total tau and tubulin are shown, and densitometry analysis of chemiluminescence signals for tau normalized to tubulin are plotted [$N = 4$ biologically independent experiments; statistical analysis is by repeated measures ANOVA, followed by Tukey's post-test (**: $p = 0.0019$)]. All bar graphs represent mean + SEM. Arrowhead on y-axis of all liquid thrashing bar graphs denotes non-Tg animals average ~70 thrashes/minute under standard laboratory conditions.

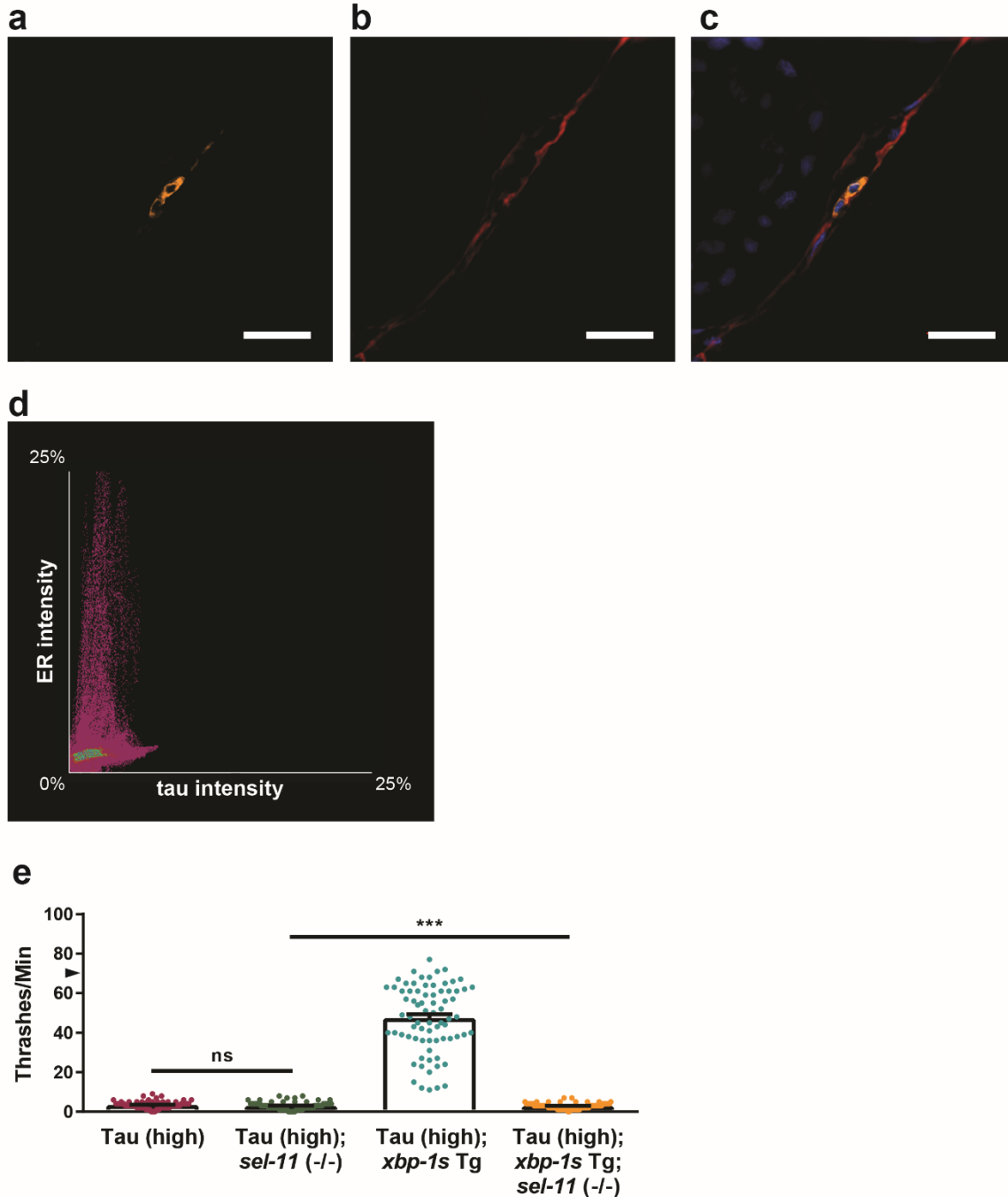


Figure 2.5: The ER does not contain tau protein, but ERAD is required for *xbp-1s*-mediated tauopathy suppression in transgenic *C. elegans*. **a-c** Tau protein does not co-localize with the ER in the Tau (high) *C. elegans* nervous system. Tau protein subcellular localization in relation to the ER integral membrane protein CP450 was measured by co-immunofluorescent staining and analyzed using structured illumination microscopy (SIM). **a** Representative SIM image of the ER protein CP450 fused to mCherry shows concentrated ER membrane localization primarily surrounding nuclei in the nerve cord [yellow = mCherry (ER); scale bar = 10 μ m]. **b** Representative SIM image of tau protein shows diffuse tau localization throughout the nerve cord (red = tau; scale bar = 10 μ m). **c** Representative merged SIM image of tau and ER membrane proteins, showing

distinct subcellular localizations [yellow = mCherry (ER); red = tau; blue = DAPI (nuclei); scale bar = 10 μ m]. **d** Colocalization analysis of ER reporter staining intensity (y-axis) with tau staining intensity (x-axis) shows no significant co-localization of the two proteins [statistical analysis is by Pearson correlation coefficient ($r = 0.23$)]. **e** *sel-11* loss of function abolishes the ability of neuronal overexpression of *xbp-1s* in Tau (high) animals to suppress severe behavioral defects observed in a liquid environment [$n = 75$ animals; $N = 5$ biologically independent experiments; bar graphs = mean + SEM; statistical analysis is by one-way ANOVA, followed by Tukey's post-test (***: $p < 0.0001$)]. Arrowhead on y-axis denotes non-Tg animals average ~70 thrashes/minute under standard laboratory conditions.

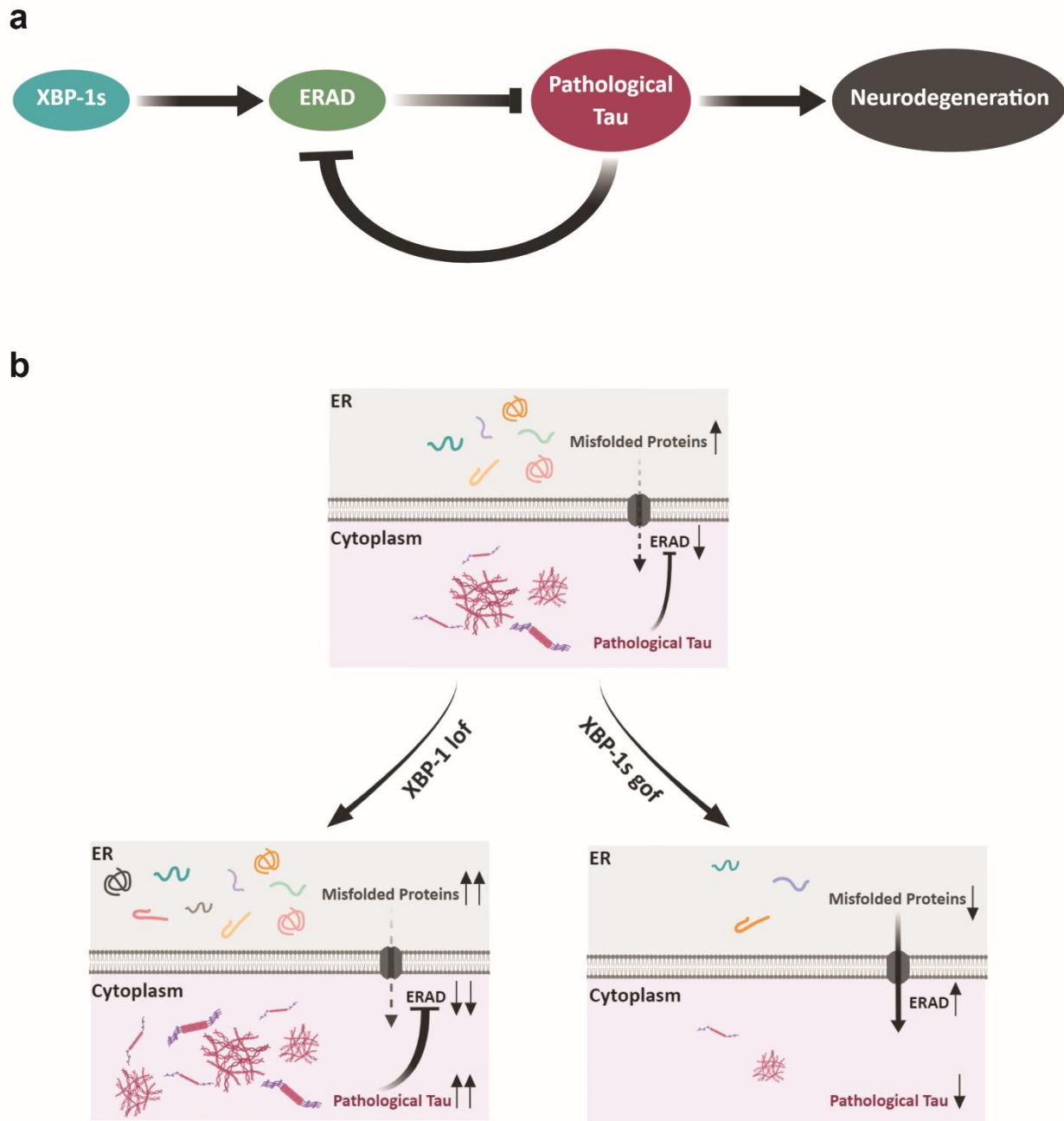
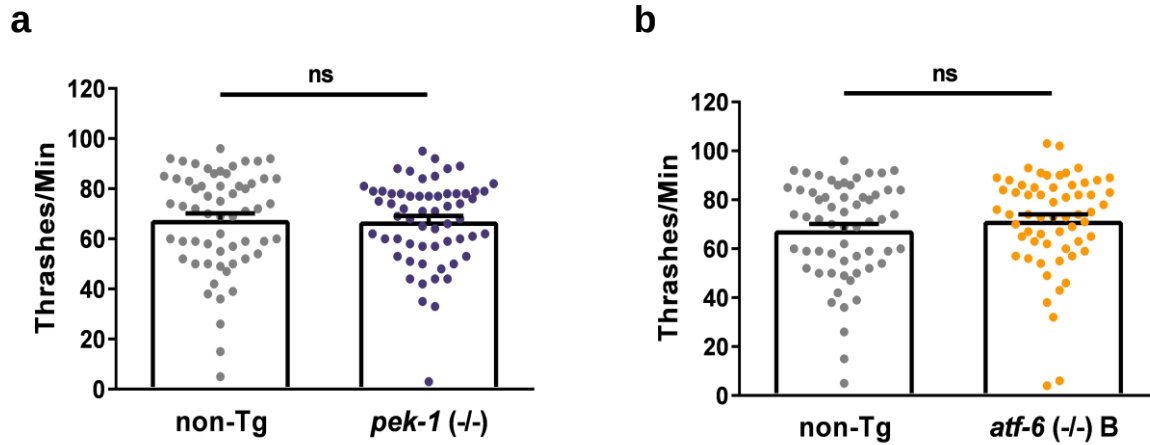
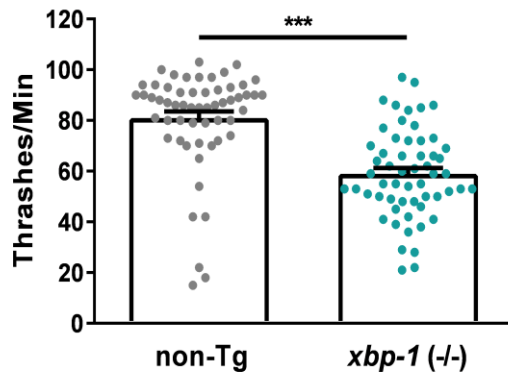


Figure 2.6: Activation of the UPR^{ER} XBP-1 transcriptional branch mediates detoxification of pathological tau via ERAD. **a** The UPR^{ER} master transcription factor XBP-1s controls transcription of ERAD genes. ERAD degrades proteins via the proteasome and autophagy, and ERAD loss of function can inhibit degradation of pathological tau. The presence of pathological tau in the cytoplasm is a stressor that impairs ERAD, leading to the accumulation of ER resident misfolded proteins and induction of the UPR^{ER} 236. **b** Loss of the UPR^{ER} XBP-1 branch promotes conversion of non-pathological tau to pathological tau, potentially by inhibition of ERAD, resulting in toxicity and subsequent neuronal cell death in *C. elegans*. Constitutive activation of the UPR^{ER} master transcription factor XBP-1s stimulates ERAD and promotes clearance of pathological tau, leading to reduction of toxicity and subsequent neuronal cell survival in *C. elegans*.

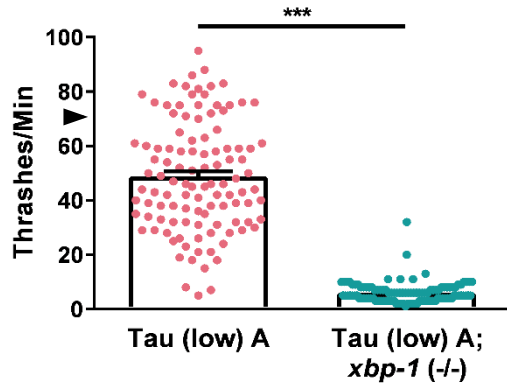
Supplementary data



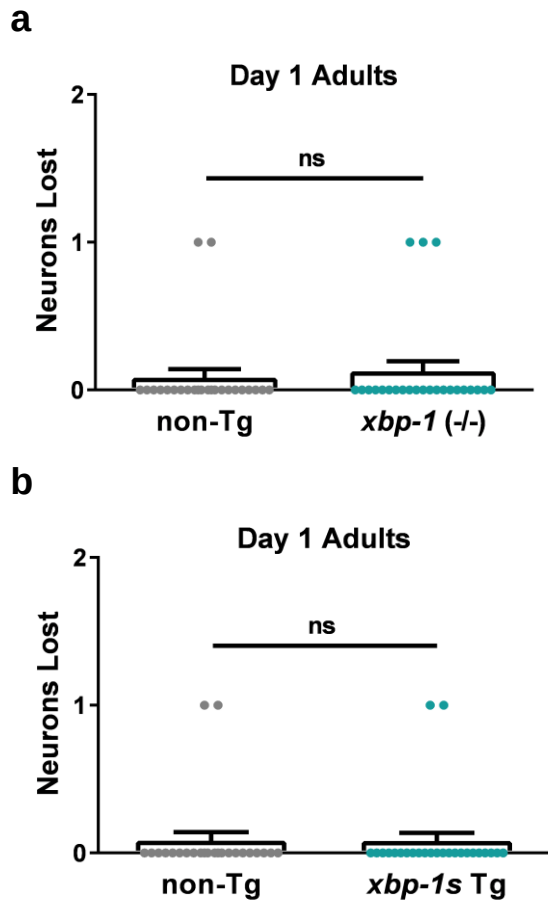
Supplementary Figure 2.1: Loss of function of the UPR^{ER} PEK-1 and ATF-6 branches does not affect locomotion in *C. elegans*. **a** Loss of *pek-1* function does not affect behavior observed in a liquid environment [$n = 60$ animals; $N = 4$ biologically independent experiments; statistical analysis is by unpaired t -test, two-tailed (ns: $p = 0.8674$)]. **b** Loss of *atf-6* function does not affect behavior observed in a liquid environment. [$n = 60$ animals; $N = 4$ biologically independent experiments; statistical analysis is by unpaired t -test, two-tailed (ns: $p = 0.2797$)]. Bar graphs represent mean + SEM.

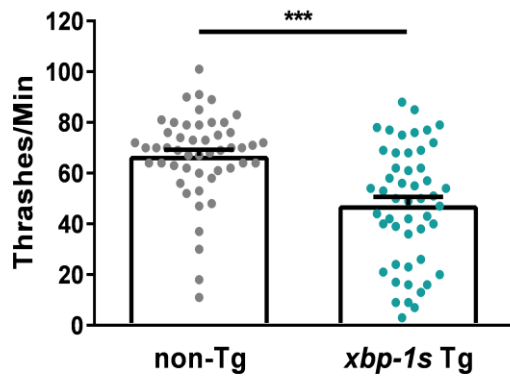


Supplementary Figure 2.2: Loss of function of the UPR^{ER} XBP-1 branch causes locomotion defects in *C. elegans*. Loss of *xbp-1* function causes mild behavioral defects observed in a liquid environment [$n = 57$ animals; $N = 3$ biologically independent experiments; statistical analysis is by unpaired t -test, two-tailed ($***p < 0.0001$)]. Bar graph represents mean + SEM.

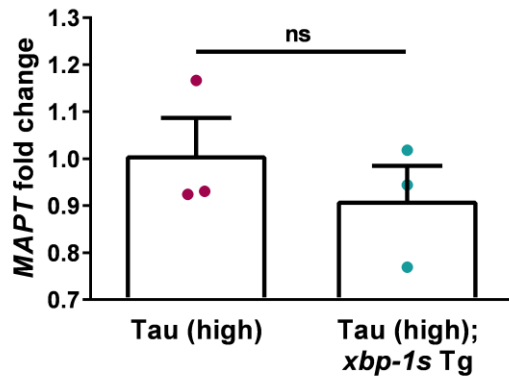


Supplementary Figure 2.3: Loss of function of the UPR^{ER} XBP-1 branch enhances locomotion defects in an independent tau transgenic *C. elegans* model. Loss of *xbp-1* function in a wildtype tau low expression background enhances mild behavioral defects observed in a liquid environment [$n = 110$ animals; $N = 7$ biologically independent experiments; statistical analysis is by unpaired t -test, two tailed ($***p < 0.0001$)]. Bar graph represents mean + SEM. Arrowhead on y-axis denotes non-Tg animals average ~70 thrashes/minute under standard laboratory conditions.

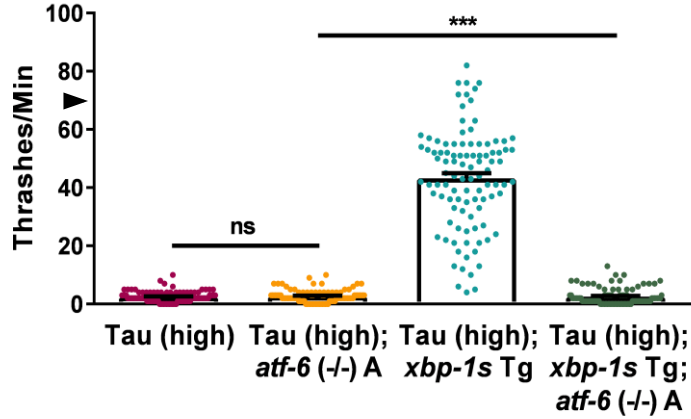
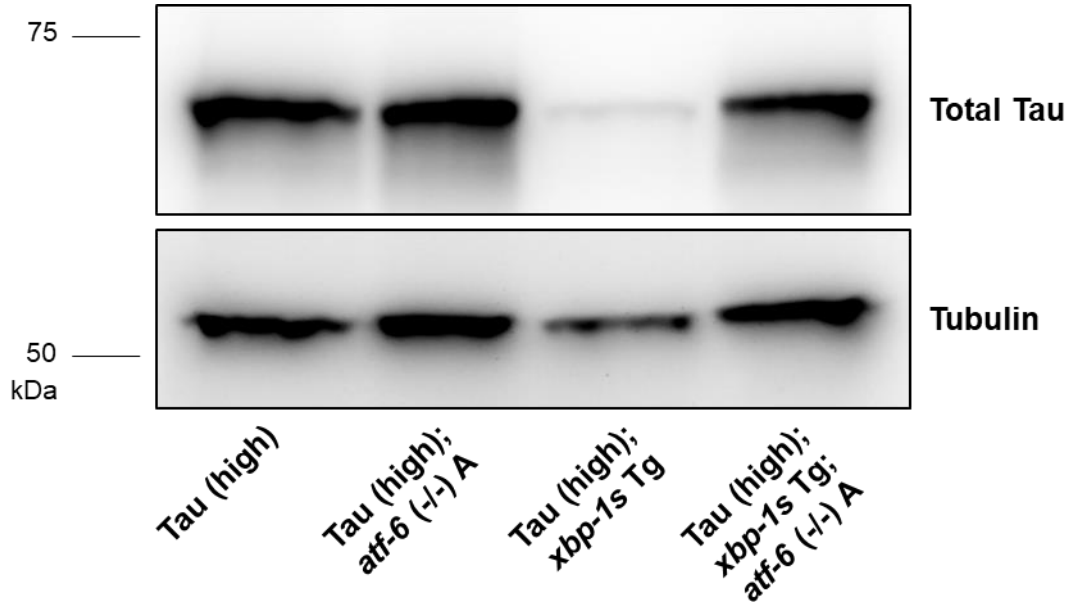




Supplementary Figure 2.5: Constitutive UPR^{ER} activation by neuronal overexpression of *xbp-1s* causes behavioral defects in *C. elegans*. Neuronal overexpression of *xbp-1s* causes mild behavioral defects observed in a liquid environment [$n = 50$ animals; $N = 3$ biologically independent experiments; statistical analysis is by unpaired t -test, two-tailed ($***p < 0.0001$)]. Bar graph represents mean + SEM.



Supplementary Figure 2.6: Constitutive UPR^{ER} activation by neuronal overexpression of *xbp-1s* does not alter human *MAPT* transcript levels in tau transgenic *C. elegans*. Neuronal overexpression of *xbp-1s* in a wildtype tau high expression background does not affect human *MAPT* gene expression levels in adult animals. *MAPT* gene expression levels normalized to *C. elegans rpl-32* gene expression levels are plotted [$N = 3$ biologically independent experiments (with three technical replicates within each experiment)]; statistical analysis is by unpaired *t*-test, two-tailed (ns: $p = 0.4250$). Bar graph represents mean + SEM.

a**b**

Supplementary Figure 2.7: Loss of function of the UPR^{ER} ATF-6 branch using an independent allele abolishes *xbp-1s*-mediated tauopathy suppression in tau transgenic *C. elegans*. **a** Loss of *atf-6* function abolishes the ability of neuronal overexpression of *xbp-1s* in a wildtype tau high expression background to suppress severe behavioral defects observed in a liquid environment [$n = 100$ animals; $N = 7$ biologically independent experiments; statistical analysis is by one-way ANOVA, followed by Tukey's post-test ($***p < 0.0001$)]. Bar graph represents mean + SEM. Arrowhead on y-axis denotes non-Tg animals average ~70 thrashes/minute under standard laboratory conditions. **b** Loss of *atf-6* function abolishes the ability of neuronal overexpression of *xbp-1s* in Tau (high) animals to decrease soluble tau protein levels. Immunoblots for total tau and tubulin are shown for one biologically independent experiment.

Supplementary Table 2.1: *C. elegans* strains

Abbreviation	Strain #	Genotype	Outcrossed	Source
non-Tg	N2	Bristol, Great Britain wildtype isolate	0x	CGC
Tau (high)	CK144	<i>aex-3p::hTau</i> (4R1N); <i>myo-2p::gfp</i>	2x	¹³¹
Tau (low) A	CK1044	<i>aex-3p::hTau</i> (4R1N); <i>myo-2p::gfp</i>	2x	¹³¹
Tau (low) B	CK1441	<i>aex-3p::hTau</i> (4R1N); <i>myo-2p::dsRED</i>	2x	This Study ¹³²
<i>xbp-1s</i> Tg	AGD927	<i>uth1s270 [rab-3p::xbp-1s; myo-2p::tdTomato]</i>	8x	¹⁹⁹
GABAergic Reporter	EG1285	<i>lin-15B</i> & <i>lin-15A(n765)</i> ; <i>ox1s12 [unc-47p::gfp] X</i>	0x	CGC
<i>pek-1</i> (-/-)	RB545	<i>pek-1(ok275) X</i>	2x	CGC
<i>atf-6</i> (-/-) A	Tm1153	<i>atf-6(tm1153) X</i>	2x	Crowder Lab
<i>atf-6</i> (-/-) B	RB772	<i>atf-6(ok551) X</i>	2x	CGC
<i>xbp-1</i> (-/-)	SJ17	<i>xbp-1(zc12) III; zcls4 [hsp-4p::gfp] V</i>	2x	CGC
<i>sel-11</i> (-/-)	MT14875	<i>nDf59 V</i>	2x	CGC
CP450 (ER)	CX10344	<i>unc-25::calf-1::gfp, unc-25::CP450::mCherry, odr-1::dsRED</i>	0x	²³²

Supplementary Table 2.2: Protein antibodies

Antigen	Clone/ Product Name	Dilution	Host Species	Source	Catalog #
β -Tubulin	E7 mAb	1:5,000	Mouse	Developmental Studies Hybridoma Bank (Iowa City, IA, USA)	N/A
Tau (Total)	SP70 pAb	1:1,000	Rabbit	Rockland Immunochemicals Inc. (Limerick, PA, USA)	200-C01-B33
pTau Ser202	CP13 mAb	1:500	Mouse	Peter Davies (Litwin-Zucker Research Center for the Study of Alzheimer's Disease, The Feinstein Institute of Medical Research, Northwell Health, Manhasset, NY, USA)	N/A
pTau Ser396/Ser404	PHF-1 mAb	1:2,000	Mouse		
pTau Ser422	EPR2866 mAb	1:500	Rabbit	Abcam (Cambridge, UK)	ab79415
pTau Thr181	AT270 mAb	1:15,000	Mouse	ThermoFisher Scientific (Waltham, MA, USA)	MN1050
Tau (Total)	K9JA (DAKO) pAb	1:250	Rabbit	Agilent Technologies, Inc. (Santa Clara, CA, USA)	A002401-2
mCherry	2F4 mAb	1:250	Mouse	Elabscience Biotechnology, Inc. (Houston, TX, USA)	E-AB-20087
2° Ab Mouse	Horseradish Peroxidase α -Ms IgG (H+L)	1:5,000	Goat	Jackson ImmunoResearch (West Grove, PA, USA)	115-035-146
2° Ab Rabbit	Horseradish Peroxidase α -Rb IgG (H+L)	1:5,000	Goat	Jackson ImmunoResearch (West Grove, PA, USA)	111-035-144
2° Ab Mouse	Alexa Fluor® 568 Goat α -Ms IgG (H+L)	1:1,000	Goat	Invitrogen (Carlsbad, CA, USA)	A-11004
2° Ab Rabbit	Alexa Fluor® 647 Goat α -Rb IgG (H+L)	1:1,000	Goat	Invitrogen (Carlsbad, CA, USA)	A-21245

Supplementary Table 2.3: Measurement of phosphorylated tau immunoblot signal

Protein	Normalized to Tubulin			Normalized to Total Tau		
	Tau (high) Mean +/- SEM (a.u.)	Tau (high); <i>xbp-1s</i> Tg Mean +/- SEM (a.u.)	<i>p</i> -value	Tau (high) Mean +/- SEM (a.u.)	Tau (high); <i>xbp-1s</i> Tg Mean +/- SEM (a.u.)	<i>p</i> -value
pTau Thr181	0.81 +/- 0.040	0.079 +/- 0.021	*** (0.0006)	0.69 +/- 0.082	0.40 +/- 0.055	* (0.0254)
pTau Ser202	0.73 +/- 0.057	0.058 +/- 0.0040	** (0.0012)	0.63 +/- 0.090	0.34 +/- 0.055	ns (0.1051)
pTau Ser396/Ser 404	0.57 +/- 0.17	0.059 +/- 0.028	* (0.0386)	0.52 +/- 0.18	0.27 +/- 0.12	* (0.0412)
pTau Ser422	0.57 +/- 0.11	0.049 +/- 0.020	* (0.0135)	0.50 +/- 0.12	0.24 +/- 0.090	* (0.0478)
Total Tau	1.21 +/- 0.14	0.18 +/- 0.042	** (0.0061)			

Densitometry analysis of chemiluminescence signals for phosphorylated tau (pTau) species normalized to tubulin and total tau ($N = 4$ biologically independent experiments; statistical analysis is by paired *t*-test, two-tailed; a.u. = arbitrary units).

CHAPTER III:

XBP-1s transcriptional reprogramming of the endoplasmic reticulum unfolded protein response modulates tauopathy

Summary

The work in this chapter is in progress for future peer-reviewed publication.

Introduction

Accumulation of unfolded proteins in the ER activates a transcriptional induction pathway known as the UPR^{ER} (**Chapter I**). Primary targets of the UPR^{ER} are molecular chaperones and folding enzymes localized in the ER; induction of these proteins augments the capacity of the protein folding system to restore ER proteostasis. In addition, some of the proteins involved in the ERAD system clear misfolded proteins from the ER and are upregulated by the UPR^{ER}. To understand transcriptional activation of the UPR^{ER}, previous work identified the *cis*-acting element and *trans*-acting factors responsible for the mammalian UPR^{ER}. The *cis*-acting ER stress response element (ERSE) with the consensus sequence CCAAT-N9-CCACG is necessary and sufficient for UPR^{ER} transcriptional induction^{249,250}. Because CCAAT of the ERSE is a binding site for the general transcription factor nuclear transcription factor Y (NF-Y)²⁵⁰, CCACG of the ERSE provides specificity to the UPR^{ER} in mammals¹⁷².

Mammalian IRE1 α is a type I ER-membrane bound endoribonuclease that initiates spliceosome-independent mRNA splicing of *XBP1* in response to ER stress²⁵¹. The spliced form of XBP1 (XBP1s) functions as a potent transcription factor via frame switch splicing, joining the bZIP and

transactivation domains¹⁷². XBP1s binds directly to the ERSE in collaboration with NF-Y to activate transcription of ER chaperone genes¹⁷².

Mammalian ATF6 is a type II transmembrane protein in the ER activated by trans-compartmental proteolysis^{188,252-254}. In response to ER stress, ER membrane-bound ATF6 is converted into the active transcription factor ATF6n in the Golgi apparatus that initiates transcription of ER chaperone genes via direct binding to the ERSE in collaboration with NF-Y; ATF6n can bind to the CCACG part of the ERSE as a homodimer or heterodimer only when NF-Y is bound to the CCAAT part^{255,256}.

The coordination of the three UPR^{ER} stress sensing and signaling pathways (PERK, IRE1 α , and ATF6) in transcriptional remodeling in tauopathy has yet to be fully investigated. Previous studies indicate the activation of both the IRE1 α /XBP1 and ATF6 pathways culminates in enhanced transcription at the ERSE site, thus upregulating the levels of ER chaperones²⁵⁷. The co-dependence of the IRE1 α and ATF6 branches merge at XBP1: *XBP1* mRNA is induced by ATF6n and spliced by IRE1 α ¹⁷², and ATF6n heterodimerizes with XBP-1s to produce more potent transcriptional activation than XBP-1s homodimers¹⁹¹. The PERK pathway is mainly responsible for translational control, but it also plays a role in transcriptional control in mammals via induction of the transcription factor ATF4. However, the ERSE is not a direct binding site of the transcription factor ATF4²⁵⁸.

Given the significance of the UPR^{ER} in aging and neurodegenerative diseases (**Chapter I**), I studied genetic manipulation of the UPR^{ER} in human wildtype tau transgenic *C. elegans* models (**Chapter II**)¹³². First, I utilized Tau (low) transgenic *C. elegans* models^{131,132} and found loss of function of XBP-1 and ATF-6 UPR^{ER} transcriptional branches enhanced tauopathy in Tau (low) backgrounds. Next, I investigated the effects of neuronal *xbp-1s* Tg *C. elegans*¹⁹⁹ in the Tau (high)

transgenic *C. elegans* model^{131,132}. XBP-1s gain of function ameliorated tauopathy phenotypes in Tau (high) transgenic *C. elegans*, which was dependent on functional ATF-6. Additionally, I showed functional ERAD via *sel-11* is required for XBP-1s-mediated tauopathy suppression in *C. elegans*. Because the two main transcriptional branches of the UPR^{ER} initiate downstream activation of genes involved in ERAD, here I conducted transcriptomic analysis of XBP-1s target genes in *C. elegans* models of tauopathy. I also utilized loss-of-function mutations and gain-of-function transgenes to genetically dissect the individual role of XBP-1s-mediated transcriptional remodeling in tau proteostasis.

Methods

Plasmids

To generate transgenes expressing HSP-4 driven by a pan-neuronal *snb-1* promoter, a double-stranded gene fragment of full-length *C. elegans hsp-4* was designed (gBlock Gene Fragment; Integrated DNA Technologies, Inc., Coralville, IA, USA). The *hsp-4* gBlock Gene Fragment served as a fragment for Gibson assembly²⁵⁹ using the *snb-1p* vector linearized with SacI and KpnI restriction enzymes.

C. elegans strains and transgenics

C. elegans strains used are listed in **Supplementary Table 3.1**. All strains were maintained at 20°C on standard nematode growth media plates containing OP50 *E. coli*¹⁰⁶. *C. elegans* were grown on NGM plates containing 5X PEP prior to collection for protein and RNA extraction. The *C. elegans* husbandry and experimentation was conducted in accordance with all relevant ethical and safety regulations for animal testing and research.

hsp-4 (high) and *hsp-4* (low) transgenic *C. elegans* strains were engineered by microinjection using a Nikon Eclipse TE300 microscope (Nikon Instruments, Inc., Melville, NY, USA) and Eppendorf FemtoJet® electronic microinjector (Eppendorf, Hamburg, Germany) setup into N2 wildtype *C. elegans* at a concentration of either 100 ng/μL *snb-1p::hsp-4*; 20 ng/μL *myo-3p::mCherry*; 50 ng/μL *UC19p* carrier DNA or 50 ng/μL *snb-1p::hsp-4*; 30 ng/μL *myo-3p::mCherry*; 70 ng/μL *UC19p* carrier DNA to produce worms carrying extrachromosomal arrays. Day one adult animals from these generated lines were irradiated for 0.8 minutes with a UV lightsource in a Stratalinker© UV Crosslinker 1800 (Stratagene, San Diego, CA, USA), after two sequential autocrosslink warm-ups to integrate the extrachromosomal array into the genome. Irradiated worms were separated onto 150 mm NGM 5X PEP plates and hard starved for several weeks. Starved populations were washed onto fresh plates and a portion of live, fluorescently marked animals were recovered to individual plates and screened for integration. Successfully integrated lines were screened by isolating individual worms with 100% transmission of the *myo-3p::mCherry* marker and outcrossed with N2 males at least two times.

C. elegans behavioral analysis

C. elegans swimming behavior was assessed as described previously¹³⁰. *C. elegans* were developmentally synchronized using a timed egg-lay and grown until all reached day one of adulthood at 20°C. Individual developmentally synchronized animals were placed in a 10 μL M9 buffer droplet on a 10 well Teflon-printed glass slide and allowed to acclimate to a liquid environment for 10 seconds. One thrash was defined as a bend across the midline or two consecutive bends from the midline toward the same side. The number of thrashes were counted during a one minute period and averaged for each strain. At least three biological replicate assay sessions with at least 10 animals per assay session were analyzed for statistical significance. I conducted behavioral analysis blinded to genotype to avoid biases. However, when the strains to be assayed were visibly distinguishable, blinding was not feasible. For comparison of two groups,

an unpaired *t*-test, two-tailed, was used. For comparisons of three or more groups, a one-way analysis of variance (ANOVA), followed by Tukey's multiple comparison post-test was used.

C. elegans protein extraction

Protein was extracted from *C. elegans* using a rigorous lysis approach¹³⁰. *C. elegans* were synchronized using hypochlorite treatment and grown until adulthood at 20°C on 5X PEP plates. Synchronized adult *C. elegans* were washed off 5X PEP plates in M9 buffer, washed an additional four times in M9 buffer to remove excess OP50 *E. coli*, pelleted by a one minute centrifugation at 800g, snap frozen in liquid nitrogen, and stored at -70°C until protein extraction. A total of two µL of high-salt reassembly (RAB) buffer (0.1 M 2-(*N*-morpholino)ethanesulfonic acid, 1 mM ethylene glycol bis-2-aminoethyl ether-*N,N',N'',n'*-tetraacetic acid, 0.5 mM MgSO₄, 0.75 M NaCl, 0.02 M NaF, pH 7.0) containing phenylmethylsulfonyl fluoride and protease inhibitors was added per milligram of packed worm pellet wet weight and homogenized via sonication four times at 70% power for eight seconds. This total soluble worm lysate was reserved for subsequent immunoblotting.

Protein immunoblotting

C. elegans protein immunoblotting was performed using Criterion apparatus (Bio-Rad) as recommended by the manufacturer (Bio-Rad Laboratories, Hercules, CA, USA)¹³⁰. *C. elegans* protein preparations were diluted 5:1 with sample buffer (0.046 M Tris, 0.005 M ethylenediamine tetraacetate, 0.2 M dithiothreitol, 50% sucrose, 5% sodium dodecyl sulfate, 0.05% bromophenol blue), boiled for 10 minutes, and centrifuged at 16,100g for two minutes. A total of 10 µL of diluted protein preparations were loaded and resolved on precast 4 to 15% gradient SDS-PAGE gel and transferred to PVDF membrane as recommended by the manufacturer (Bio-Rad Laboratories, Hercules, CA, USA). PVDF membranes were blocked in 5% milk in PBS for one hour before

overnight incubation with primary antibody at 4°C. The next day, PVDF membranes were washed in PBS with 0.05% Tween, incubated at room temperature with HRP-coupled secondary antibody for two hours, and washed in PBS with 0.05% Tween before visualization. The dilutions and pertinent details for all primary and secondary antibodies used are listed in **Supplementary Table 3.2**. Enhanced chemiluminescence substrate (Bio-Rad Laboratories, Hercules, CA, USA) was added to the PVDF membrane, and chemiluminescence signals were detected with ChemiDoc-It®² 510 Imager (UVP LLC, Upland, CA, USA). Relative intensity of chemiluminescence signals was measured with ImageJ Java²³⁰. Three biological assay replicates were analyzed for statistical significance. For comparison of four groups run on an independent immunoblot, a repeated measures ANOVA, followed by Tukey's multiple comparison post-test was used.

C. elegans RNA purification

To achieve a tightly synchronized *C. elegans* population, two rounds of synchronization were completed. First, mixed population *C. elegans* were synchronized using hypochlorite treatment and grown until adulthood at 20°C on 5X PEP plates. From this adult population, *C. elegans* were synchronized using hypochlorite treatment and grown until L2 larval stage at 20°C on 5X PEP plates. Synchronized L2 *C. elegans* were washed off 5X PEP plates in M9 buffer, washed an additional four times in M9 buffer to remove excess OP50 *E. coli*, pelleted by a one minute centrifugation at 800g, snap frozen in liquid nitrogen, and stored at -70°C until RNA extraction. RNA was purified from snap-frozen packed *C. elegans* pellets using TRIzol Reagent as directed by the manufacturer's instructions²³³. RNA was resuspended in 50 uL sterile water. RNA concentration and purity were assessed using a NanoPhotometer® NP80 spectrophotometer (Implen GmbH, Munich, Germany). RNA integrity was assessed by 1% TBE agarose gel electrophoresis.

Library construction and transcriptomic analysis

C. elegans cDNA libraries for next generation sequencing were prepared from purified total RNA using the TruSeq reagents (Illumina, Inc., San Diego, CA, USA) at the Northwest Genomics Center within the University of Washington Department of Genome Sciences. Libraries were sequenced using Illumina NovaSeq technology (Illumina, Inc., San Diego, CA, USA) with at least 25 million reads per sample. Three biological replicates with two technical replicates for each sample were analyzed. Transcriptomic analysis was completed using the Lasergene bioinformatics software version 17 (DNASTAR, Inc., Madison, WI, USA). The sequencing data was aligned to the *C. elegans* genome (release WB235) using Lasergene SeqMan NGen software (DNASTAR, Inc., Madison, WI, USA). Subsequent differential gene expression and Gene Ontology analysis was completed using Lasergene ArrayStar software (DNASTAR, Inc., Madison, WI, USA). Comparison of non-Tg and *xbp-1s* Tg animals yielded 2,697 upregulated genes with two-fold or greater differential gene expression levels in *xbp-1s* Tg versus non-Tg animals. Among these genes, 560 genes exhibited significant gene expression changes in all four groups, analyzed by a two-way ANOVA, followed by a Benjamini-Hochberg post-test, with 131 of these genes exhibiting an F-value greater than 10. Filtering this gene set for genes with *xbp-1s* levels greater than 0.1 Reads Per Kilobase Million (RPKM) yielded a total of 116 significant genes in this analysis (**Table 3.1**). The group of *xbp-1s* target genes was analyzed using the Basic Local Alignment Search Tool (BLAST)²⁶⁰ to identify human orthologs (BLAST expected values $< 1 \times 10^{-6}$), yielding 11 significant genes (**Table 3.2**) and 5 significant genes with literature evidence of an association with ATF6 (**Table 3.3**).

Statistical analysis

Statistical significance for all phenotype assays was determined using GraphPad Prism statistical software (GraphPad Software, Inc., La Jolla, CA, USA). Statistical significance is demarcated in figures as *: $p < 0.05$, **: $p < 0.005$, and ***: $p < 0.0001$.

Results

Transcriptomic analysis reveals candidate XBP-1s target genes involved in tauopathy suppression

The IRE1 α /XBP1 pathway is the most conserved branch of the UPR^{ER}, leading to the expression of the master UPR^{ER} transcription factor XBP1s^{165,166}. Previously, I demonstrated XBP-1s overexpression in neurons can rescue tauopathy in *C. elegans* (**Chapter II**)¹³². Because XBP-1s is an active transcription factor, I hypothesized XBP-1s transcriptional changes may contribute to tauopathy amelioration in *C. elegans*. To test this hypothesis, I again utilized the pan-neuronal Tau (high) transgenic *C. elegans* model, which exhibits severe behavioral phenotypes and accumulation of pathological tau protein^{131,132}. I crossed Tau (high) animals with pan-neuronal *xbp-1s* gain-of-function transgenic animals (*xbp1-s* Tg), which promotes transcriptional activation of the XBP-1s UPR^{ER} branch in the absence of ER stress¹⁹⁹. Transcriptomic analysis was conducted of young *C. elegans* at the L2 larval stage when neurons contribute the largest relative mass and cell number per animal. I isolated RNA from large, synchronized L2 populations of non-Tg, *xbp-1s* Tg, Tau (high), and Tau (high); *xbp-1s* Tg animals and sequenced it using Illumina based sequencing technology. Transcriptomic analysis of the sequenced RNA populations revealed 116 genes with robust and statistically significant expression changes (i.e., at least two-fold change in *xbp-1s* Tg versus non-Tg). These XBP-1s target gene candidates for mediating

tauopathy suppression are shown in **Table 3.1** and served as the basis for identifying novel regulators of tauopathy.

The three UPR^{ER} branches exert unique and coordinated transcriptional and translational remodeling to restore proteostasis, with the IRE1α/XBP1 and ATF6 branches controlling the majority of transcriptional changes. In *C. elegans*, my previous work revealed *xbp-1s*-mediated tauopathy suppression requires a functional ATF-6 branch (**Chapter II**)¹³², and other studies suggest the ATF-6n transcriptional pathway might have evolved as a backup mechanism to the XBP-1s transcriptional pathway¹⁸⁰. In mammals, ATF6n and XBP1s can form transcriptionally active heterodimers, which exhibit a higher binding affinity to target genes than XBP1s homodimers^{191,192}. I hypothesized the gene(s) responsible for rescue of tauopathy in *C. elegans* might be responsive to both XBP-1s and ATF-6n. To examine this possibility, I first analyzed the XBP-1s target genes identified in **Table 3.1** for conservation between *C. elegans* and humans, revealing 11 genes (**Table 3.2**). Next, I investigated the conserved XBP1s target genes for literature evidence of ATF6n responsiveness (**Table 3.3**). From this analysis, I identified five genes exhibiting significant responsiveness to XBP-1s in the transcriptomic data that also have a previously demonstrated functional gene classification association with ATF6n: caspase 1 (*csp-1*)²⁶¹, DNAJ domain (prokaryotic heat shock protein) 28 (*dnj-28*)²⁶², heat shock protein 4 (*hsp-4*)^{188,263}, choline kinase beta 2 (*ckb-2*)²⁶⁴, and lipase like 3 (*lipl-3*)²⁶⁵.

A suite of ATF6 dependent XBP-1s target genes are required for tauopathy suppression

To validate the transcriptional analysis of XBP-1s responsive genes in *C. elegans* with a known association with ATF6 (**Table 3.3**), I examined available *csp-1*, *dnj-28*, *hsp-4*, *ckb-2*, and *lipl-3* loss-of-function mutant strains in the Tau (high); *xbp-1s* Tg *C. elegans* background. I hypothesized if these five target genes are necessary, genetic loss of function might alter *xbp-1s*-mediated tauopathy suppression in *C. elegans*.

The *csp-1* gene in *C. elegans* is orthologous to the cysteine-aspartic acid protease (caspase; CASP) family of genes in humans, such as *CASP1* and *CASP3*, *CASP6*, and *CASP7*²⁶⁶. Caspases are protease enzymes playing essential roles in programmed cell death, or apoptosis. The mechanism of apoptosis is evolutionarily conserved, and the critical involvement of the caspase cell death abnormality 3 (CED-3) protein in apoptosis was first discovered in *C. elegans*²⁶⁷. In mammals, 14 caspases have currently been identified and are functionally divided into three subfamilies: apoptosis initiator (*CASP2*, *CASP8*, *CASP9*, *CASP10*), apoptosis effector (*CASP3*, *CASP6*, *CASP7*), and inflammatory mediator (*CASP1*, *CASP4*, *CASP5*, *CASP11*, *CASP12*, *CASP13*, *CASP14*)^{268,269}.

The consequence of caspase activity loss of function in *xbp-1s*-mediated tauopathy suppression was analyzed using a *C. elegans* mutant strain [*csp-1* (-/-)]. Tau (high); *xbp-1s* animals were crossed with *csp-1* (-/-) animals to understand the effect on tauopathy behavioral phenotypes. When I examined the deletion of *csp-1* in Tau (high) animals, I found there was no change in tau-induced behavioral dysfunction when compared to Tau (high) animals alone (**Figure 3.1**). As I have shown previously (**Chapter II**)¹³², Tau (high); *xbp-1s* Tg animals exhibited robust rescue of the tau-mediated locomotion defects compared to Tau (high) animals alone (**Figure 3.1**). Interestingly, *csp-1* (-/-) loss of function abolished the ability of *xbp-1s* to suppress locomotion defects as seen in Tau (high); *xbp-1s* Tg; *csp-1* (-/-) animals compared to Tau (high); *xbp-1s* Tg animals (**Figure 3.1**).

C. elegans dnj-28 is orthologous to human *DNAJC3*, which encodes the ER stress-regulated chaperone DnaJ heat shock protein family (Hsp40) member C3 (*DNAJC3*), also known as 58-kilodalton inhibitor of protein kinase (P58^{IPK}). The transcription of *DNAJC3* is induced in response to ER stress²⁷⁰. *DNAJC3* has diverse functions in the ER, including being a co-chaperone and

regulator of BiP²⁷¹⁻²⁷⁴ and inhibition of PERK activity to relieve the PERK-mediated translational attenuation²⁷⁰.

I investigated the effects of *dnj-28* loss of function in *xbp-1s*-mediated tauopathy suppression in *C. elegans* by using a loss-of-function mutant strain [*dnj-28 (-/-)*]. Tau (high); *xbp-1s* Tg animals were crossed with *dnj-28 (-/-)* animals, and I measured the effect on tauopathy behavioral phenotypes. Deletion of *dnj-28* in Tau (high) animals did not change the tau-induced locomotion abnormalities when compared to Tau (high) animals alone (**Figure 3.2**). When I tested whether *dnj-28 (-/-)* could prevent *xbp-1s* suppression of tau-induced behavioral abnormalities, I found *dnj-28* loss of function was necessary for *xbp-1s* Tg to suppress behavioral defects in Tau (high); *xbp-1s* Tg; *dnj-28 (-/-)* animals when compared to Tau (high); *xbp-1s* Tg animals (**Figure 3.2**).

The *C. elegans* gene *hsp-4* is an ortholog of the human heat shock protein A5 (*HSPA5*), which encodes the ER-resident molecular chaperone commonly known as BiP. As one of the most abundant proteins in the ER, BiP maintains ER homeostasis via a variety of functions, including protein folding processes, protein import into the ER, regulation of calcium homeostasis, and facilitation of ERAD²⁷⁵. Under ER stress conditions, BiP can initiate the UPR^{ER}, decrease unfolded protein load, induce autophagy, and crosstalk with apoptosis machinery to assist in the cell survival decision²⁷⁵.

To understand the potential role of BiP in *xbp-1s*-mediated tauopathy suppression in *C. elegans*, I utilized a *hsp-4* loss-of-function mutant strain [*hsp-4 (-/-)*]. When compared to non-Tg animals, *hsp-4 (-/-)* animals had mild locomotion defects (**Supplementary Figure 3.1**). Tau (high); *xbp-1s* Tg animals were crossed with *hsp-4 (-/-)* animals, and I evaluated the effect on tauopathy phenotypes. I tested consequences of deleting *hsp-4* in Tau (high) animals and observed no change in tau-induced behavioral dysfunction when compared to Tau (high) animals alone

(**Figure 3.3a**). Finally, I tested whether *hsp-4* (-/-) could prevent *xbp-1s*-mediated suppression of tau-induced behavioral abnormalities. I found *hsp-4* loss of function fully blocked the ability of *xbp-1s* to suppress behavioral defects in Tau (high); *xbp-1s* Tg; *hsp-4* (-/-) animals (**Figure 3.3a**). However, it is possible this amelioration of *xbp-1s*-mediated tauopathy suppression may be due to the movement abnormalities displayed in *hsp-4* (-/-) animals (**Supplementary Figure 3.1**).

Because *xbp-1s*-mediated tauopathy behavioral suppression requires *hsp-4*, I also measured *hsp-4* loss of function effects on total tau protein by immunoblot. Loss of function of *hsp-4* alone did not change tau protein levels in Tau (high) animals compared to Tau (high) animals alone (**Figure 3.3b, c**). As I have shown previously (**Chapter II**)¹³², *xbp-1s* gain of function reduced tau accumulation in the Tau (high) background (**Figure 3.3b, c**). Interestingly, Tau (high); *xbp-1s* Tg; *hsp-4* (-/-) animals accumulated total tau protein comparable to the level seen in Tau (high) animals (**Figure 3.3b, c**). Taken together, these data demonstrate *hsp-4* loss of function significantly impacts the ability of *xbp-1s* gain of function to suppress behavioral and biochemical tauopathy phenotypes observed in the *C. elegans* Tau (high) background.

C. elegans ckb-2 is orthologous to human choline kinase alpha and beta genes (*CHKA* and *CHKB*, respectively). Human choline kinase alpha (ChoK α) and beta (ChoK β) are members of a family of enzymes involved in the regulation of lipid metabolism²⁷⁶. ChoK catalyzes the first reaction in the choline pathway for phosphatidylcholine biosynthesis, which plays a critical role in membrane structure and cell signaling²⁷⁶. Using a *ckb-2* loss-of-function allele, I attempted to generate Tau (high); *xbp-1s* Tg; *ckb-2* (-/-) animals. However, *ckb-2* and the neuronal *xbp-1s* transgene both reside on *C. elegans* chromosome III, and I was unable to investigate CKB-2 function in *xbp-1s*-mediated tauopathy suppression.

The *lipl-3* gene in *C. elegans* is orthologous to a human class of genes known as lipase (LIP) family members, including *LIPA*, *LIPJ*, *LIPF*, *LIPK*, *LIPN*²⁶⁶, and *LIPM*. The *LIPA* gene exhibits the highest homology to *lipl-3* and encodes the lysosomal acid lipase (LAL) enzyme found in lysosomal compartments, which is essential for lipid metabolism. LAL breaks down lipids such as cholesterol esters and triglycerides to generate free fatty acids and cholesterol²⁷⁷.

To understand whether functional LIPL-3 is necessary for *xbp-1s*-mediated tauopathy suppression, I used a loss-of-function mutant strain [*lipl-3* (-/-)]. I crossed Tau (high); *xbp-1s* Tg animals with *lipl-3* (-/-) animals and explored the effect on tauopathy locomotion defects. Loss of function of *lipl-3* did not significantly affect Tau (high) locomotion defects (**Figure 3.4**). In contrast, Tau (high); *xbp-1s* Tg; *lipl-3* (-/-) animals displayed similar locomotion abnormalities when compared to Tau (high) animals alone (**Figure 3.4**), indicating *lipl-3* is also required for *xbp-1s*-mediated tauopathy suppression in *C. elegans*. Taken altogether, four genes identified as responsive to XBP-1s in the transcriptomic data with a previously known association with ATF6n are all essential for *xbp-1s*-mediated tauopathy suppression in *C. elegans*. These results support the robustness of the XBP-1s neuronally-enriched transcriptional analysis in *C. elegans* and provide information on novel potential mediators of tauopathy.

UPR^{ER} non-responsive homolog of HSP-4 does not modulate tauopathy phenotypes

To further validate the XBP-1s transcriptional analysis of potential genes involved in tauopathy suppression in *C. elegans*, I investigated whether another member of the same functional gene classification could ameliorate *xbp-1s*-mediated tauopathy suppression. Given the significance of BiP in the UPR^{ER} and the evidence *hsp-4* loss of function can suppress the effect of *xbp-1s* Tg on behavioral and biochemical tauopathy phenotypes in Tau (high) *C. elegans* (**Figure 3.3**), I explored the consequences of *hsp-3* loss of function. In *C. elegans*, HSP-3 and HSP-4 are homologous to mammalian BiP. HSP-3 is both constitutively expressed and stress-responsive,

while HSP-4 has very low basal expression in most cells but is strongly induced by UPR^{ER} signaling^{245,278}. Tau (high); *xbp-1s* Tg animals were crossed with a *C. elegans hsp-3* mutant strain [*hsp-3* (-/-)] and I measured the effect on locomotion. Tau (high); *hsp-3* (-/-) animals displayed a similar defect in locomotion when compared to Tau (high) animals alone (**Figure 3.5**). When compared to Tau (high); *xbp-1s* Tg animals, Tau (high); *xbp-1s* Tg; *hsp-3* (-/-) animals exhibited similar suppression of tauopathy-induced locomotion defects (**Figure 3.5**), indicating *hsp-3* loss of function does not impact the ability of *xbp-1s* gain of function to suppress tauopathy-induced behavioral defects. Although HSP-3 and HSP-4 proteins are highly conserved and thought to be functionally redundant²⁷⁸, I have shown *xbp-1s*-mediated tauopathy suppression in *C. elegans* is specific, requiring functional HSP-4 (**Figure 3.3**).

ATF6 dependent XBP-1s target genes differentially affect tauopathy phenotypes

Given the importance of four genes identified in the *C. elegans* transcriptomic data exhibiting significant responsiveness to XBP-1s and associated with ATF6n (**Table 3.3**), I hypothesized loss of function of these target genes might alter tauopathy phenotypes. Because the severity of behavioral dysfunction of Tau (high) animals might affect my ability to discriminate enhancement of tauopathy phenotypes, I utilized the pan-neuronal Tau (low) transgenic *C. elegans* model, which exhibits mild behavioral deficits and no significant accumulation of pathological tau species^{131,132}. I crossed Tau (low) animals with either *dnj-28* or *hsp-4* loss-of-function mutant strains and measured the consequence on tau-induced behavioral dysfunction.

If *dnj-28* and *hsp-4* regulate tau proteostasis in *C. elegans*, I expected Tau (low) animals crossed with either loss-of-function mutant strain would exhibit enhancement of tau-dependent locomotion dysfunction. However, when crossed with Tau (low) animals, *dnj-28* (-/-) did not worsen Tau (low) behavioral defects (**Figure 3.6a**), suggesting DNJ-28 function alone is not required for tauopathy suppression in *C. elegans*. Next, I tested whether *hsp-4* loss of function could modulate the mild

tauopathy locomotion phenotype seen in Tau (low) Tg animals. Contrastingly, Tau (low); *hsp-4* (-/-) animals displayed significant enhancement of the locomotion defects seen in Tau (low) Tg worms alone (**Figure 3.6b**). In agreement with my previous findings in *C. elegans* regarding functional ATF-6, HSP-4 function appears to be essential in tauopathy, although the enhancement of tauopathy behavioral phenotypes is not as strong as XBP-1s loss of function (**Chapter II**)¹³².

Neuronal hsp-4 activation modulates tauopathy phenotypes

Given HSP-4 loss of function blocks *xbp-1s*-mediated tauopathy suppression of behavioral and biochemical phenotypes in the Tau (high) background (**Figure 3.3**) and enhances tauopathy phenotypes in the Tau (low) background (**Figure 3.6b**), I hypothesized *hsp-4* gain of function alone might play a role in tauopathy in *C. elegans*. To investigate this possibility, our laboratory generated pan-neuronal *hsp-4* transgenic *C. elegans* lines with varying expression levels using a different neuronal promoter than those driving Tau (high) and *xbp-1s* Tg *C. elegans* expression. Both neuronal low overexpression [*hsp-4* (low) Tg] and high overexpression [*hsp-4* (high) Tg A and B] *C. elegans* models were generated. I crossed *hsp-4* Tg animals with Tau (high) animals and analyzed the effect on tau-induced locomotion defects. When compared to Tau (high) animals alone, Tau (high); *hsp-4* (high) Tg A and B animals exhibited similar tau-induced behavioral defects (**Figure 3.7**). Interestingly, low overexpression of *hsp-4* was able to mildly suppress behavioral tauopathy phenotypes, as seen in Tau (high); *hsp-4* (low) Tg animals compared to Tau (high) animals alone (**Figure 3.7**). These results indicate HSP-4 function can mediate tauopathy suppression in *C. elegans* but requires a fine-tuned expression level.

Discussion

Aging-related declines in cellular function and neurodegenerative diseases are intimately associated with overall cellular proteostasis, including the UPR^{ER}. Much work has been conducted to understand the role of the UPR^{ER} in the context of health and disease over the lifespan. However, contradictory results in neurodegenerative tauopathies (**Chapter I**) warrants a more direct approach to dissect the underlying mechanisms of the UPR^{ER} in tauopathy. Because the three stress transducers of the UPR^{ER} each control transcriptional regulation via XBP1s, ATF6n, and ATF4 transcription factors, understanding the role of downstream upregulated target genes is important for identifying unique regulators of tauopathy. Here, I followed up on my previous study (**Chapter II**)¹³² by exploring the role of UPR^{ER} transcriptional regulation in human wildtype tau transgenic *C. elegans*.

Constitutive activation of the master UPR^{ER} transcription factor XBP-1s suppresses tauopathy phenotypes in *C. elegans*, which relies on functional ATF-6 (**Chapter II**)¹³². Because XBP-1s is essential for UPR^{ER} transcriptional remodeling, I conducted whole organism transcriptomic analysis at the L2 developmental stage to enrich for neuronal transcripts upregulated by XBP-1s in Tau (high) *C. elegans*. I narrowed my search to find candidate XBP-1s target genes in three sequential steps: identifying significant genes upregulated in *xbp-1s* Tg animals compared to non-Tg animals (116 genes; **Table 3.1**), identifying significant genes with human homologs (11 genes; **Table 3.2**), and identifying significant genes with human homologs and a known association with ATF6 (5 genes; **Table 3.3**). From this analysis, I nominated five genetic modifiers of tauopathy associated with XBP-1s/ATF6 in *C. elegans*: *csp-1*, *dnj-28*, *hsp-4*, *ckb-2*, and *lip1-3*.

Another study conducted intestine-specific RNA sequencing analysis in *C. elegans* to identify genes upregulated in the intestine when XBP-1s is overexpressed in neurons through non-cell autonomous UPR^{ER} signalling²⁷⁹. Neuronal XBP-1s significantly increased the expression of

genes involved in lysosome function, and intestinal lysosome function was necessary for enhanced lifespan and proteostasis²⁷⁹. Consistent with my results regarding the lipase family member *lipl-3* gene, *lipl-1* and *lipl-5* were significantly upregulated in the intestine in response to neuronal *xbp-1s* Tg overexpression²⁷⁹. Additionally, *dnj-28*, *hsp-4*, and *ckb-2* were also upregulated in this RNA sequencing dataset²⁷⁹. However, the caspase family of genes was not identified as significantly upregulated or downregulated²⁷⁹. This difference indicates a potential divergence of neuronal XBP-1s in tauopathy suppression versus lifespan extension, which warrants further investigation.

To genetically dissect the contributions of each XBP-1s/ATF6 candidate gene, I systematically eliminated the function of *csp-1*, *dnj-28*, *hsp-4*, *ckb-2*, and *lipl-3* in Tau (high); *xbp-1s* Tg *C. elegans*. I supported the validity of the transcriptomic analysis by showing loss of function of four candidate genes abolished *xbp-1s*-mediated tauopathy suppression in *C. elegans* (**Figures 3.1-4**). However, I was unable to investigate CKB-2 loss of function in the Tau (high); *xbp-1s* Tg *C. elegans* background due to close proximity on chromosome III of the *ckb-2* loss-of-function allele and integrated neuronal *xbp-1s* transgene.

To fully understand the specificity of these five nominated genes in *C. elegans* tauopathy, I tested whether another member of the same functional gene classification affected Tau (high); *xbp-1s* Tg animal phenotypes. In contrast to *hsp-4* loss of function (**Figure 3.3**), *hsp-3* (-/-) did not affect *xbp-1s*-mediated behavioral tauopathy suppression (**Figure 3.5**). Future studies will need to address whether *dnj-28*, *csp-1*, and *lipl-3* gene classification control genes can also block the ability of XBP-1s to ameliorate tauopathy phenotypes in *C. elegans*. The DNAJ domain class of genes in *C. elegans* includes 29 genes²⁶⁶, and I propose investigation of *dnj-27* loss of function in *xbp-1s*-mediated tauopathy suppression in *C. elegans*. When *dnj-27* expression is downregulated by RNAi in human A β , alpha-synuclein (α -synuclein), and polyglutamine (polyQ)

transgenic *C. elegans* models, there is enhancement of neurodegenerative phenotypes²⁸⁰. The *C. elegans* lipase like gene class includes eight genes²⁶⁶, and I propose future studies should examine *lip1-1* and/or *lip1-5* loss of function in Tau (high); *xbp-1s* Tg *C. elegans*. Neuronal overexpression of *xbp-1s* upregulates *lip1-1* and *lip1-5* expression in the intestine, which is essential for lifespan extension; however, *lip1-3* was not identified as a significant gene in this study²⁷⁹. Four genes are members of the caspase gene class in *C. elegans*²⁶⁶, and I propose investigation of the role of *ced-3* loss of function in *xbp-1s*-mediated tauopathy amelioration in *C. elegans*. CED-3 is the major programmed cell death caspase expressed in *C. elegans* neurons, and previous work indicates neuronal dysfunction caused by human wildtype and mutant transactive response DNA-binding protein 43 (TDP-43) in *C. elegans* does not require functional CED-3²⁸¹. Taken altogether, it is necessary to understand whether the three remaining target genes identified in Tau (high); *xbp-1s* Tg *C. elegans* are specific to tauopathy or whether tauopathy suppression via XBP-1s is generalizable to the function of each gene classification system.

Finally, I tested whether the five nominated XBP-1s/ATF6 target genes could modulate tauopathy in *C. elegans*. I found *hsp-4* loss of function enhanced behavioral tauopathy phenotypes, while *dnj-28* loss of function does not regulate tauopathy in Tau (low) animals (**Figure 3.6**). Our laboratory also generated neuronal *hsp-4* overexpression transgenic *C. elegans* models and I crossed these with Tau (high) animals. I showed low expression of *hsp-4* modestly suppresses tauopathy behavioral phenotypes, while *hsp-4* Tg high expression had no effect (**Figure 3.7**). Given the intricate nature of XBP-1s-mediated transcriptional remodeling, future work should explore loss of function and gain of function of all five candidate target genes in tauopathy suppression. This will provide an overall mechanistic understanding of XBP-1s transcriptional regulation in *C. elegans* tauopathy models and potential direct therapeutic targets for modulating tauopathy in humans.

Data

Table 3.1: XBP-1s responsive genes identified by RNAseq in *C. elegans*

Worm Gene	RNA Fold Change [Tau (high); <i>xbp-1s</i> Tg vs. Tau (high)]	RNA Fold Change [<i>xbp-1s</i> Tg vs. non-Tg]	RNA Fold Change [Tau (high) vs. non-Tg]
<i>acr-18</i>	7.001 ↑	3.132 ↑	1.135 ↑
<i>B0041.11</i>	4.629 ↑	3.152 ↑	1.300 ↑
<i>C01B4.6</i>	1.337 ↑	3.394 ↑	1.002 ↓
<i>C01B4.7</i>	1.059 ↓	2.751 ↑	1.529 ↑
<i>C01B4.8</i>	1.040 ↓	2.555 ↑	1.120 ↑
<i>C04F12.1</i>	8.173 ↑	10.040 ↑	1.748 ↑
<i>C08F1.11</i>	1.099 ↓	2.638 ↑	5.787 ↑
<i>C08F1.13</i>	2.027 ↑	32.560 ↑	18.763 ↑
<i>C08F1.8</i>	1.412 ↑	2.420 ↑	3.405 ↑
<i>C09H10.4</i>	4.482 ↑	4.163 ↑	1.100 ↑
<i>C13B7.6</i>	1.038 ↓	2.524 ↑	1.132 ↑
<i>C15H7.4</i>	2.048 ↑	2.737 ↑	4.480 ↑
<i>C17B7.12</i>	1.693 ↑	3.292 ↑	1.518 ↓
<i>C17B7.15</i>	2.989 ↑	3.600 ↑	1.828 ↓
<i>C17B7.2</i>	1.992 ↑	3.054 ↑	1.467 ↓
<i>C17B7.4</i>	2.022 ↑	4.937 ↑	1.068 ↑
<i>C18D4.6</i>	1.447 ↑	2.000 ↑	2.396 ↑
<i>C36B7.2</i>	11.316 ↑	4.716 ↑	3.157 ↑
<i>C36C5.5</i>	1.619 ↑	3.004 ↑	3.997 ↓
<i>C38D9.2</i>	1.478 ↑	3.279 ↑	4.260 ↑
<i>C38D9.5</i>	3.006 ↑	4.023 ↑	4.379 ↑
<i>C44H9.4</i>	4.429 ↑	10.979 ↑	9.040 ↑
<i>ceh-83</i>	1.822 ↑	2.307 ↑	4.201 ↑
<i>ckb-2</i>	1.948 ↑	2.151 ↑	1.539 ↑
<i>cllec-209</i>	1.076 ↓	17.775 ↑	2.236 ↑
<i>cllec-241</i>	34.518 ↑	97.237 ↑	0
<i>cllec-47</i>	2.304 ↑	2.637 ↑	1.515 ↑
<i>csp-1</i>	6.650 ↑	10.596 ↑	4.312 ↑
<i>dnj-28</i>	5.698 ↑	6.711 ↑	1.627 ↑
<i>eol-1</i>	1.004 ↓	2.034 ↑	2.899 ↑
<i>F17B5.1</i>	1.624 ↑	2.600 ↑	1.789 ↑
<i>F19G12.3</i>	4.134 ↑	2.306 ↑	2.130 ↑
<i>F22E5.6</i>	1.373 ↑	3.374 ↑	7.739 ↑
<i>F31E9.6</i>	1.688 ↑	2.382 ↑	2.637 ↑
<i>F35F10.5</i>	3.265 ↑	5.416 ↑	1.888 ↓
<i>F35F10.6</i>	1.511 ↑	3.064 ↑	1.137 ↓
<i>F35F10.7</i>	2.284 ↑	3.954 ↑	1.115 ↑

<i>F36A2.14</i>	2.299 ↑	12.434 ↑	23.155 ↑
<i>F36D3.16</i>	67.208 ↑	23.291 ↑	1.329 ↑
<i>F36H5.10</i>	1.727 ↑	3.579 ↑	2.882 ↑
<i>F40G12.5</i>	5.321 ↑	5.072 ↑	2.520 ↑
<i>F40G12.6</i>	4.637 ↑	2.676 ↑	1.344 ↑
<i>F41B4.3</i>	3.336 ↑	2.865 ↑	1.467 ↑
<i>F41E7.6</i>	11.090 ↑	4.315 ↑	1.352 ↓
<i>F42A6.3</i>	13.334 ↑	6.713 ↑	1.113 ↓
<i>F42G8.7</i>	17.065 ↑	3.980 ↑	1.000 ↓
<i>F44G3.2</i>	1.251 ↓	2.356 ↑	3.833 ↑
<i>F47H4.2</i>	1.531 ↑	3.314 ↑	3.595 ↑
<i>F54H12.8</i>	7.864 ↑	16.881 ↑	6.898 ↑
<i>F56A4.10</i>	1.126 ↑	5.391 ↑	1.048 ↑
<i>F56A4.12</i>	1.104 ↓	2.684 ↑	1.208 ↑
<i>F56A4.2</i>	1.106 ↓	17.041 ↑	2.068 ↑
<i>F56A4.3</i>	1.057 ↑	3.798 ↑	1.011 ↑
<i>fbxa-11</i>	1.301 ↑	2.009 ↑	1.865 ↑
<i>fbxa-170</i>	2.587 ↑	26.705 ↑	36.269 ↑
<i>fbxa-185</i>	2.137 ↑	5.825 ↑	9.050 ↑
<i>fbxa-189</i>	1.187 ↓	2.271 ↑	4.395 ↑
<i>fbxa-52</i>	1.462 ↑	2.378 ↑	3.578 ↑
<i>fbxb-53</i>	8.010 ↑	16.789 ↑	4.023 ↑
<i>fbxb-87</i>	1.313 ↑	28.049 ↑	53.551 ↑
<i>H12D21.2</i>	7.438 ↑	3.097 ↑	2.005 ↓
<i>H36L18.2</i>	1.087 ↓	2.269 ↑	3.714 ↓
<i>hke-4.1</i>	4.252 ↑	4.354 ↑	1.262 ↑
<i>hsp-4</i>	3.262 ↑	2.911 ↑	1.083 ↑
<i>ilys-3</i>	1.100 ↑	3.082 ↑	9.152 ↑
<i>irld-35</i>	3.854 ↑	33.231 ↑	6.106 ↓
<i>K09C6.9</i>	1.719 ↑	2.145 ↑	2.818 ↑
<i>K09F6.10</i>	2.478 ↓	2.573 ↑	20.105 ↑
<i>K09F6.13</i>	1.219 ↓	2.639 ↑	6.765 ↑
<i>K10G4.5</i>	1.495 ↑	15.726 ↑	23.944 ↑
<i>linc-64</i>	3.296 ↑	17.558 ↑	20.083 ↑
<i>lip1-3</i>	1.766 ↑	18.205 ↑	20.347 ↑
<i>lys-10</i>	1.737 ↑	3.098 ↑	7.183 ↑
<i>M04G7.5</i>	491.954 ↑	32.282 ↑	5.879 ↓
<i>mct-2</i>	1.320 ↓	2.917 ↑	1.716 ↑
<i>nas-10</i>	1.671 ↓	2.009 ↑	4.690 ↑
<i>nhr-222</i>	6.549 ↑	3.337 ↑	1.172 ↓
<i>rrf-2</i>	1.543 ↑	2.228 ↑	3.487 ↑
<i>sdz-35</i>	1.325 ↓	3.254 ↑	10.259 ↑
<i>srbc-15</i>	0	5882.456 ↑	0

<i>srh-179</i>	105.663 ↑	295.893 ↑	10.925 ↑
<i>srh-181</i>	782.717 ↑	1155.178 ↑	4.327 ↑
<i>str-199</i>	2.208 ↑	27.042 ↑	11.884 ↑
<i>T18D3.14</i>	1.525 ↑	125.641 ↑	1258.439 ↑
<i>T20D4.10</i>	2.887 ↑	4.257 ↑	2.663 ↓
<i>T20D4.11</i>	2.757 ↑	3.312 ↑	3.419 ↓
<i>T20D4.12</i>	3.441 ↑	7.424 ↑	2.712 ↓
<i>T20D4.20</i>	2.243 ↑	2.902 ↑	1.678 ↓
<i>T28A11.2</i>	1.965 ↑	2.766 ↑	1.220 ↓
<i>T28A11.25</i>	1.703 ↑	3.405 ↑	1.064 ↑
<i>tsp-4</i>	5.421 ↓	4.359 ↑	52.812 ↑
<i>Y116F11B.9</i>	664.407 ↑	251.487 ↑	1.073 ↓
<i>Y19D10A.16</i>	1.026 ↑	3.061 ↑	1.198 ↑
<i>Y19D10A.4</i>	1.128 ↑	3.505 ↑	1.867 ↑
<i>Y19D10A.5</i>	1.050 ↓	3.116 ↑	1.319 ↑
<i>Y19D10A.8</i>	1.029 ↓	5.335 ↑	1.002 ↓
<i>Y20C6A.1</i>	1.089 ↓	2.235 ↑	3.795 ↑
<i>Y37H2A.16</i>	1.019 ↑	2.140 ↑	3.401 ↑
<i>Y38F1A.1</i>	3.025 ↑	2.457 ↑	1.306 ↑
<i>Y43F8B.23</i>	1.747 ↑	2.139 ↑	1.870 ↑
<i>Y45G12C.16</i>	1.108 ↓	2.532 ↑	1.172 ↑
<i>Y51B9A.8</i>	1.471 ↑	2.689 ↑	2.800 ↓
<i>Y53G8AR.1</i>	89.441 ↑	100.965 ↑	2.266 ↑
<i>Y53G8B.3</i>	2.221 ↑	15.614 ↑	7.966 ↑
<i>Y68A4A.12</i>	3.190 ↑	31.429 ↑	22.300 ↑
<i>Y68A4A.13</i>	1.044 ↑	5.021 ↑	12.496 ↑
<i>Y71A12B.19</i>	2.491 ↑	2.264 ↑	2.484 ↑
<i>Y71A12B.8</i>	51.270 ↑	50.274 ↑	3.316 ↑
<i>Y82E9BL.9</i>	1.036 ↓	2.013 ↑	2.353 ↓
<i>Y82E9BR.1</i>	1.145 ↑	2.486 ↑	2.708 ↑
<i>ZC239.5</i>	2.805 ↑	4.844 ↑	1.858 ↑
<i>ZC404.15</i>	3.377 ↑	3.220 ↑	1.413 ↑
<i>ZC412.9</i>	8.920 ↑	2.433 ↑	2.778 ↓
<i>ZK218.1</i>	2.814 ↑	2.232 ↑	2.412 ↑
<i>ZK218.3</i>	26.075 ↑	79.883 ↑	7.562 ↑
<i>ZK218.5</i>	5.180 ↑	9.955 ↑	2.345 ↑

Table 3.2: XBP-1s responsive genes with human orthologs identified by RNAseq in *C. elegans*

Worm Gene	Human Gene	Protein Description (Location)	RNA Fold Change		
			Tau (high); <i>xbp-1s</i> Tg vs. Tau (high)	<i>xbp-1s</i> Tg vs. non-Tg	Tau (high) vs. non-Tg
<i>F42G8.7 (erp-44.3)</i>	<i>ERP44</i>	Protein Disulfide Isomerase (ER)	17.065 ↑	3.980 ↑	1.000 ↓
<i>F41E7.6</i>	<i>CROT</i>	Carnitine O-Octanoyltransferase (Peroxisome)	11.090 ↑	4.315 ↑	1.352 ↓
<i>csp-1</i>	<i>CASP14</i>	Aspartyl Protease (Nucleus)	6.650 ↑	10.596 ↑	4.312 ↑
<i>dnj-28</i>	<i>DNAJC3</i>	DNAJ, co-chaperone of HSP70 (ER)	5.698 ↑	6.711 ↑	1.627 ↑
<i>hsp-4</i>	<i>HSPA5/ BiP/ GRP-78</i>	HSP70, chaperone (ER)	3.262 ↑	2.911 ↑	1.083 ↑
<i>ckb-2</i>	<i>CHKA/CHKB</i>	Choline Kinase (Cytoplasm)	1.948 ↑	2.151 ↑	1.539 ↑
<i>lipl-3</i>	<i>LIP</i> family	Lipase (Lysosome)	1.766 ↑	18.205 ↑	20.347 ↑
<i>C01B4.6</i>	<i>GALM</i>	Galactose Mutarotase (Cytoplasm)	1.337 ↑	3.394 ↑	1.002 ↓
<i>Y19D10A.16</i>	<i>GALM</i>	Galactose Mutarotase (Cytoplasm)	1.026 ↑	3.061 ↑	1.198 ↑
<i>eol-1</i>	<i>DXO</i>	Decapping Exoribonuclease (Nucleus)	1.004 ↓	2.034 ↑	2.899 ↑
<i>mct-2</i>	<i>SLC16A14</i>	Carnitine Transporter (Plasma Membrane)	1.320 ↓	2.917 ↑	1.716 ↑

Table 3.3: XBP-1s responsive genes associated with ATF6 identified by RNAseq in *C. elegans*

Worm Gene	Human Gene	Protein Description (Subcellular Location)	RNA Fold Change [Tau (high); <i>xbp-1s</i> Tg vs. Tau (high)]	ATF6 Association
<i>csp-1</i>	<i>CASP14</i>	Aspartyl Protease (Nucleus)	6.650 ↑	261
<i>dnj-28</i>	<i>DNAJC3</i>	DNAJ, co-chaperone of HSP70 (ER)	5.698 ↑	262
<i>hsp-4</i>	<i>HSPA5/BiP/ GRP-78</i>	HSP70, chaperone (ER)	3.262 ↑	188,263
<i>ckb-2</i>	<i>CHKA/CHKB</i>	Choline Kinase (Cytoplasm)	1.948 ↑	264
<i>lip1-3</i>	<i>LIP</i> family	Lipase (Lysosome)	1.766 ↑	265

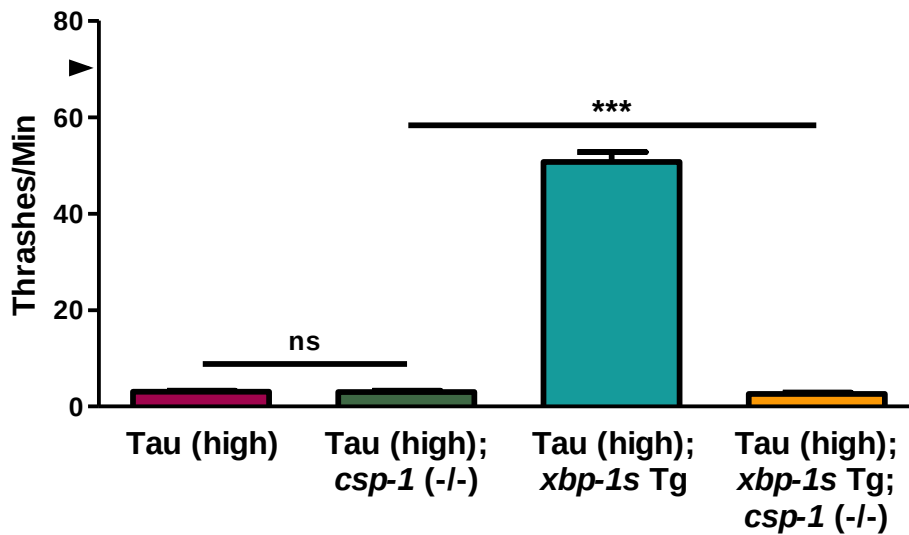


Figure 3.1: Cytoplasmic resident CSP-1 caspase protein induction by XBP-1s/ATF6 is required for suppression of tauopathy in transgenic *C. elegans*. *csp-1* loss of function abolishes the ability of neuronal overexpression of *xbp-1s* in Tau (high) animals to suppress severe behavioral defects observed in a liquid environment [$n = 75$ animals; $N = 5$ biologically independent experiments; statistical analysis is by one-way ANOVA, followed by Tukey's post-test (***: $p < 0.0001$)]. Bar graph represents mean + SEM. Arrowhead on y-axis denotes non-Tg animals average ~70 thrashes/minute under standard laboratory conditions.

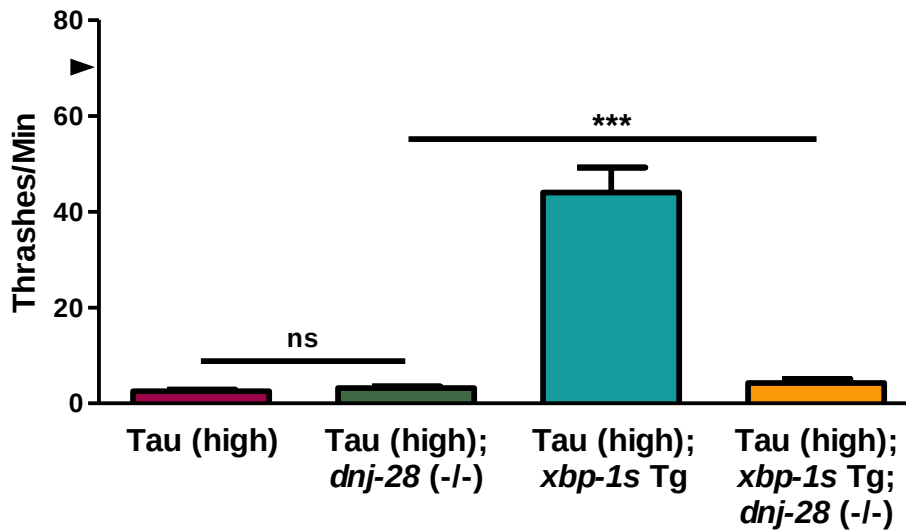
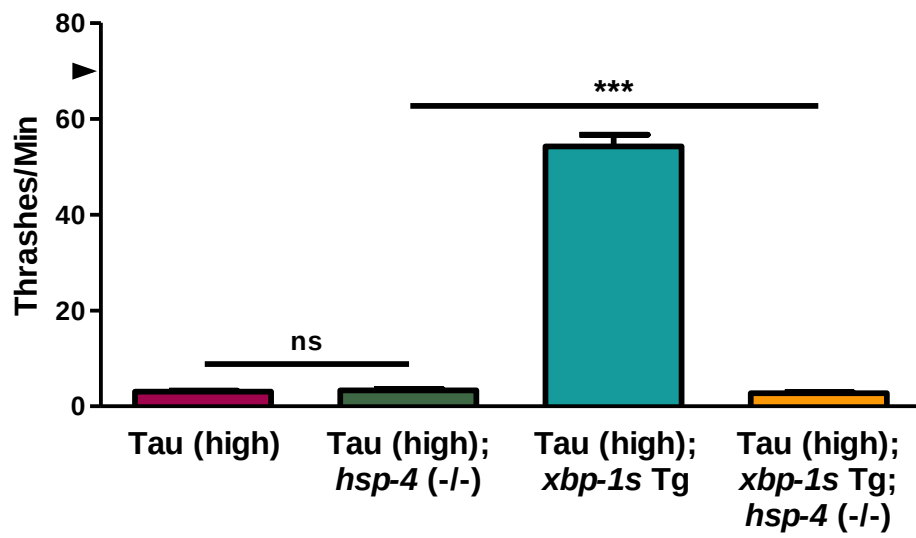
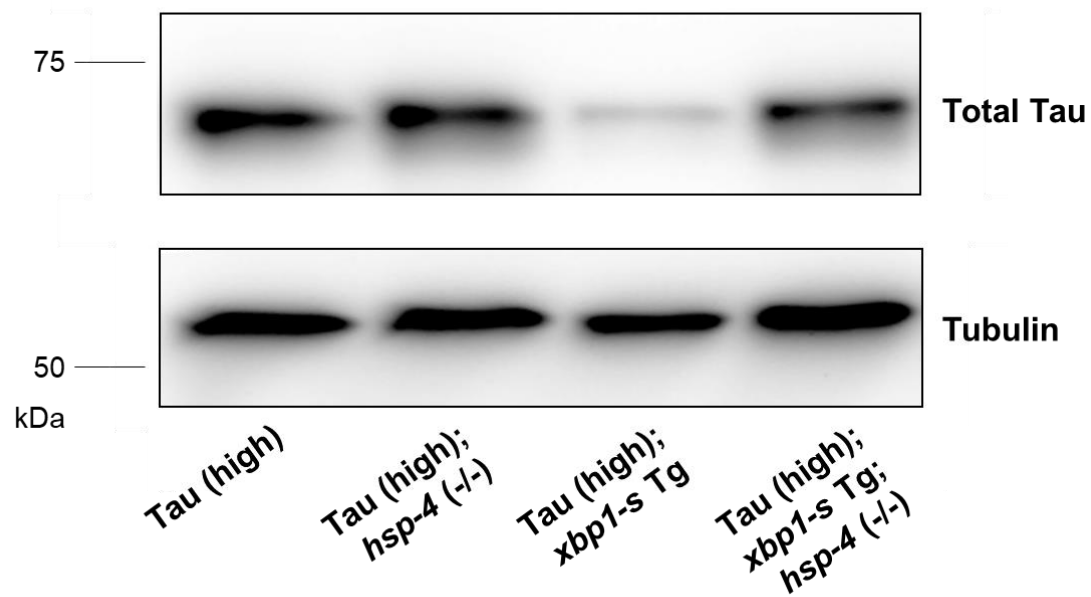


Figure 3.2: ER resident DNJ-28 co-chaperone heat shock protein induction by XBP-1s/ATF6 is required for suppression of tauopathy in transgenic *C. elegans*. *dnj-28* loss of function abolishes the ability of neuronal overexpression of *xbp-1s* in Tau (high) animals to suppress severe behavioral defects observed in a liquid environment [$n = 97$ animals; $N = 7$ biologically independent experiments; statistical analysis is by one-way ANOVA, followed by Tukey's post-test (***: $p < 0.0001$)]. Bar graph represents mean + SEM. Arrowhead on y-axis denotes non-Tg animals average ~70 thrashes/minute under standard laboratory conditions.

a



b



C

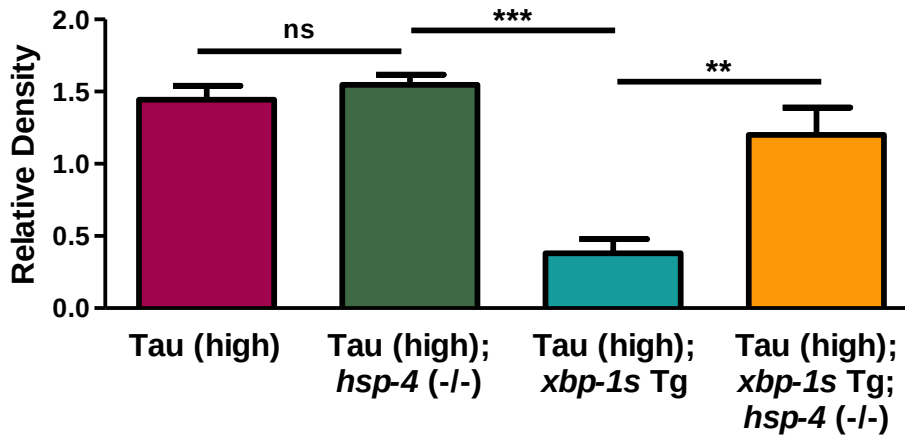


Figure 3.3: ER resident HSP-4 heat shock protein induction by XBP-1s/ATF6 is required for suppression of tauopathy in transgenic *C. elegans*. **a** *hsp-4* loss of function abolishes the ability of neuronal overexpression of *xbp-1s* in Tau (high) animals to suppress severe behavioral defects observed in a liquid environment [$n = 90$ animals; $N = 6$ biologically independent experiments; statistical analysis is by one-way ANOVA, followed by Tukey's post-test (***: $p < 0.0001$)]. **b, c** *hsp-4* loss of function abolishes the ability of neuronal overexpression of *xbp-1s* in Tau (high) animals to decrease soluble tau protein levels. Representative immunoblots for total tau and tubulin are shown, and densitometry analysis of chemiluminescence signals for tau normalized to tubulin are plotted [$N = 3$ biologically independent experiments; statistical analysis is by repeated measures ANOVA, followed by Tukey's post-test (***: $p = 0.0009$)]. All bar graphs represent mean + SEM. Arrowhead on y-axis of liquid thrashing bar graph denotes non-Tg animals average ~70 thrashes/minute under standard laboratory conditions.

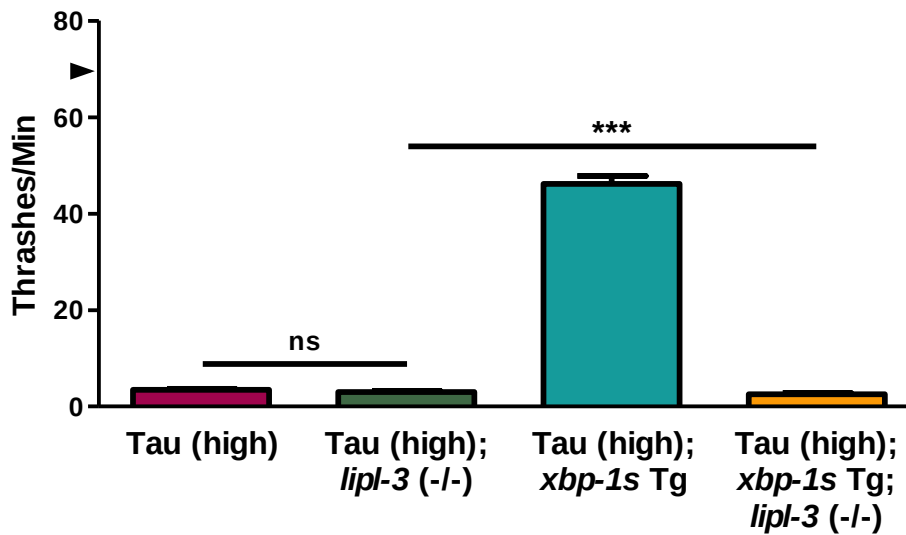


Figure 3.4: Lysosomal resident LIPL-3 lipase protein induction by XBP-1s/ATF6 is required for suppression of tauopathy in transgenic *C. elegans*. *lip1-3* loss of function abolishes the ability of neuronal overexpression of *xbp-1s* in Tau (high) animals to suppress severe behavioral defects observed in a liquid environment [$n = 90$ animals; $N = 6$ biologically independent experiments; statistical analysis is by one-way ANOVA, followed by Tukey's post-test (***: $p < 0.0001$)]. Bar graph represents mean + SEM. Arrowhead on y-axis denotes non-Tg animals average ~70 thrashes/minute under standard laboratory conditions.

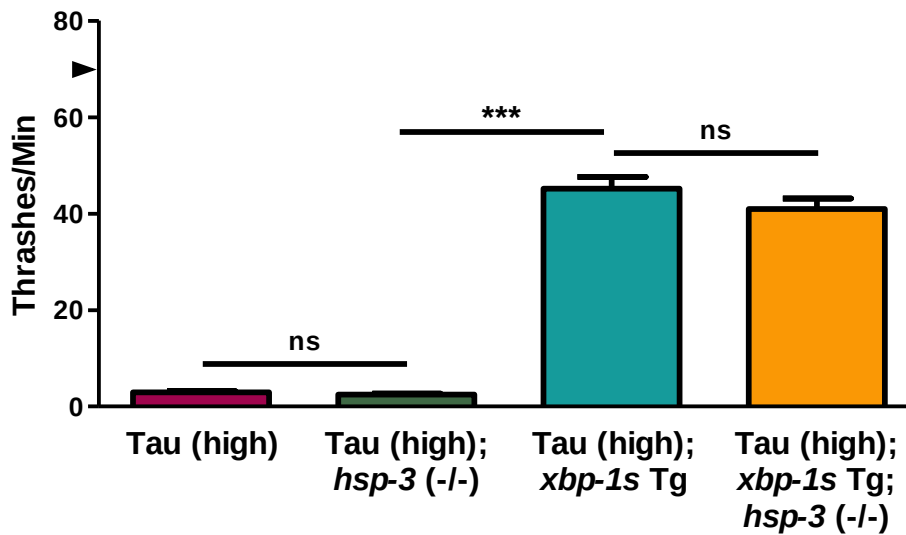


Figure 3.5: ER resident HSP-3 heat shock protein induction by XBP-1s/ATF6 is dispensable for suppression of tauopathy in transgenic *C. elegans*. *hsp-3* loss of function does not alter the ability of neuronal overexpression of *xbp-1s* in Tau (high) animals to suppress severe behavioral defects observed in a liquid environment [$n = 60$ animals; $N = 4$ biologically independent experiments; statistical analysis is by one-way ANOVA, followed by Tukey's post-test (***: $p < 0.0001$)]. Bar graph represents mean + SEM. Arrowhead on y-axis denotes non-Tg animals average ~70 thrashes/minute under standard laboratory conditions.

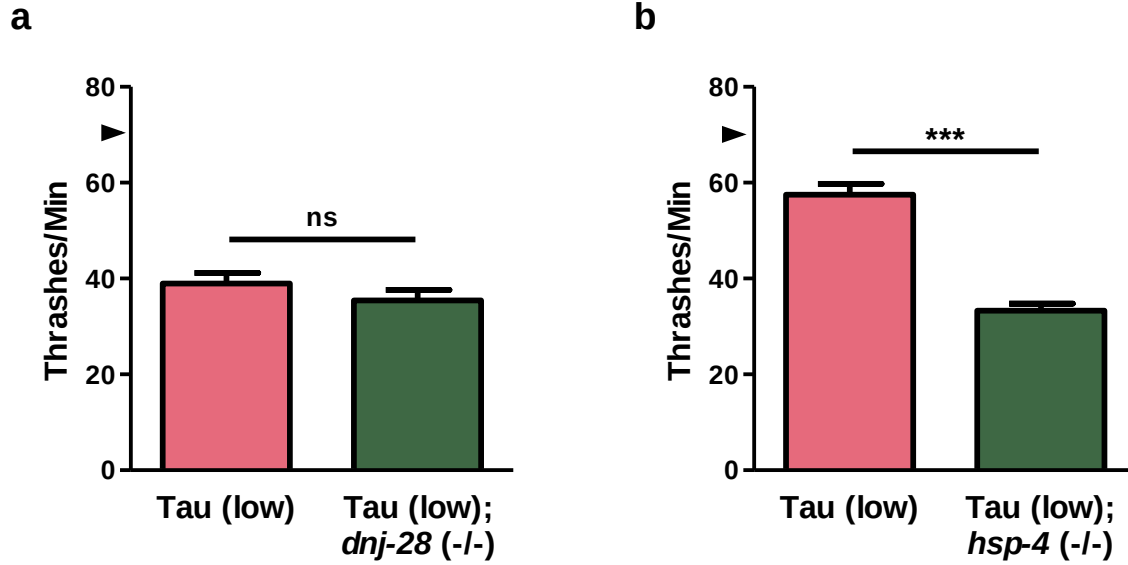


Figure 3.6: XBP-1s/ATF6 target gene induction is differentially involved in tauopathy in transgenic *C. elegans*. **a** *dnj-28* loss of function in Tau (low) animals does not alter mild behavioral defects observed in a liquid environment [$n = 96$ and 94 animals, respectively; $N = 5$ biologically independent experiments; statistical analysis is by unpaired t -test, two tailed (ns: $p = 0.2586$)]. **b** *hsp-4* loss of function in Tau (low) animals enhances mild behavioral defects observed in a liquid environment [$n = 100$ animals; $N = 5$ biologically independent experiments; statistical analysis is by t -test, two tailed (***: $p < 0.0001$)]. All bar graphs represent mean + SEM. Arrowhead on y-axis denotes non-Tg animals average ~70 thrashes/minute under standard laboratory conditions.

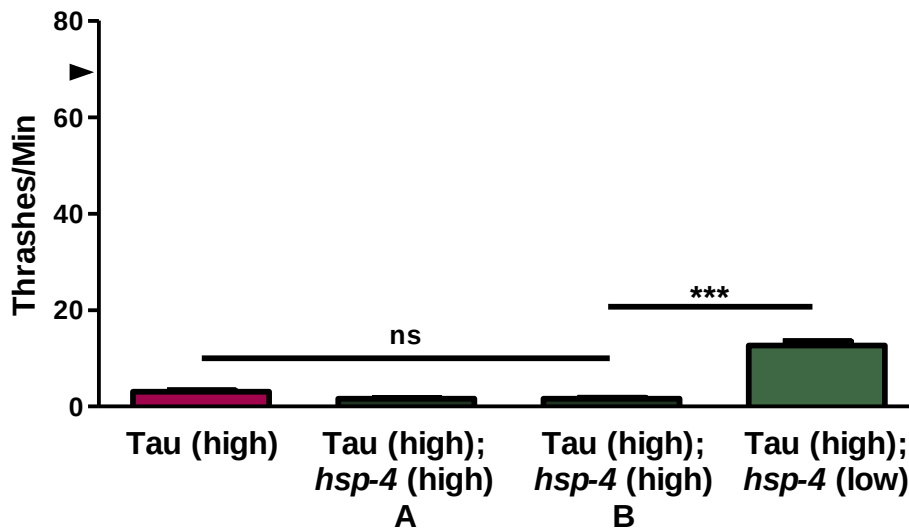
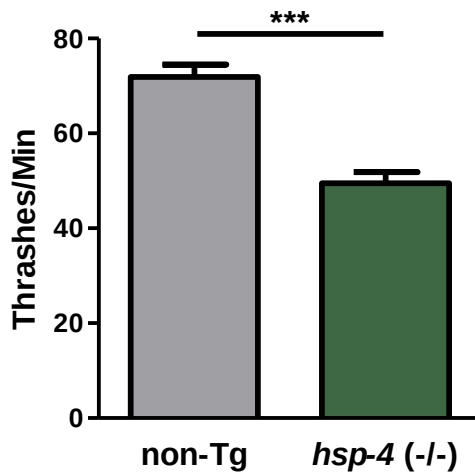


Figure 3.7: Neuronal low overexpression of ER resident HSP-4 heat shock protein modulates tauopathy in transgenic *C. elegans*. Neuronal high overexpression of *hsp-4* in Tau (high) animals does not alter severe behavioral defects, while neuronal low overexpression of *hsp-4* in Tau (high) animals mildly suppresses severe behavioral defects observed in a liquid environment [$n = 70, 70,$ and 69 animals, respectively; $N = 5$ biologically independent experiments; statistical analysis is by one-way ANOVA, followed by Tukey's post-test (***: $p < 0.0001$)]. Bar graph represents mean + SEM. Arrowhead on y-axis denotes non-Tg animals average ~70 thrashes/minute under standard laboratory conditions.

Supplementary data



Supplementary Figure 3.1: Loss of function of ER resident HSP-4 heat shock protein causes locomotion defects in *C. elegans*. Loss of *hsp-4* function causes mild behavioral defects observed in a liquid environment [$n = 30$ animals; $N = 2$ biologically independent experiments; statistical analysis is by unpaired t -test, two-tailed ($***p < 0.0001$)]. Bar graph represents mean + SEM.

Supplementary Table 3.1: *C. elegans* strains

Abbreviation	Strain #	Genotype	Outcrossed	Source
non-Tg	N2	Bristol, Great Britain wildtype isolate	0x	CGC
Tau (high)	CK144	<i>aex-3p::hTau</i> (4R1N); <i>myo-2p::gfp</i>	2x	131,132
Tau (low)	CK1044	<i>aex-3p::hTau</i> (4R1N); <i>myo-2p::gfp</i>	2x	131,132
<i>xbp-1s</i> Tg	AGD927	<i>uth1s270</i> [<i>rab-3p::xbp-1s</i> ; <i>myo-2p::tdTomato</i>]	8x	199
<i>csp-1</i> (-/-)	RB1954	<i>Y48E1B.13(ok2570)</i> II	2x	CGC
<i>dnj-28</i> (-/-)	RB1917	<i>dnj-28(ok2490)</i> I	2x	CGC
<i>hsp-4</i> (-/-)	VC1099	<i>hsp-4(gk514)</i> II	3x	CGC
<i>ckb-2</i> (-/-)	RB1577	<i>ckb-2(ok1922)</i> III	2x	CGC
<i>lip1-3</i> (-/-)	VC40849	<i>lip1-3(gk846191)</i> V	2x	CGC
<i>hsp-3</i> (-/-)	RB1104	<i>hsp-3(ok1083)</i> X	2x	CGC
<i>hsp-4</i> (low) Tg	CK1911	<i>snb-1p::hsp-4</i> ; <i>myo-3p::mCherry</i>	3x	This Study
<i>hsp-4</i> (high) Tg A	CK1785	<i>snb-1p::hsp-4</i> ; <i>myo-3p::mCherry</i>	2x	This Study
<i>hsp-4</i> (high) Tg B	CK1786	<i>snb-1p::hsp-4</i> ; <i>myo-3p::mCherry</i>	2x	This Study

Supplementary Table 3.2: Protein antibodies

Antigen	Clone/ Product Name	Dilution	Host Species	Source	Catalog #
β -Tubulin	E7 mAb	1:5,000	Mouse	Developmental Studies Hybridoma Bank (Iowa City, IA, USA)	N/A
Tau (Total)	SP70 pAb	1:1,000	Rabbit	Rockland Immunochemicals Inc. (Limerick, PA, USA)	200-C01-B33
2° Ab Mouse	Horseradish Peroxidase α - Ms IgG (H+L)	1:5,000	Goat	Jackson ImmunoResearch (West Grove, PA, USA)	115-035-146
2° Ab Rabbit	Horseradish Peroxidase α - Rb IgG (H+L)	1:5,000	Goat	Jackson ImmunoResearch (West Grove, PA, USA)	111-035-144

CHAPTER IV:

Conclusions and future directions

The UPR^{ER} is involved in tauopathy in *C. elegans*

The UPR^{ER} is an evolutionarily conserved mechanism essential for restoring imbalances in ER proteostasis and present in single-celled organisms, invertebrates, and mammals (**Chapter I**). In multicellular eukaryotes, the UPR^{ER} is comprised of three branches, including IRE1 α , PERK, and ATF6. IRE1 α and ATF6 UPR^{ER} stress transducers control transcriptional remodeling via XBP1s and ATF6n transcription factors, respectively. While the PERK branch also exhibits transcriptional control through the ATF4 transcription factor, the main function of this UPR^{ER} transducer is to attenuate overall protein synthesis to reduce additional ER protein folding load. Initial activation of the UPR^{ER} attempts to ameliorate ER stress; under prolonged stress conditions, the UPR^{ER} induces cell death through CHOP to mediate overall tissue viability. Aging drives a decline in proteostasis capacity, which can adversely affect post-mitotic neurons and contribute to neurodegenerative disease pathology, such as tauopathies (**Chapter I**). Using a genetically tractable model system, I first explored the functional role of the three UPR^{ER} branches in *C. elegans* transgenic models of human tauopathy (**Chapter II**). I showed *pek-1* loss of function does not regulate tauopathy phenotypes, while both *xbp-1* and *atf-6* loss of function enhance tauopathy phenotypes (**Chapter II**). These findings indicate UPR^{ER} transcriptional regulation may be an important mediator of tauopathy in *C. elegans* models.

Transcriptional activation of the UPR^{ER} modulates tauopathy suppression in *C. elegans*

Although all three UPR^{ER} branches initiate downstream transcriptional changes to restore ER proteostasis, the IRE1 α /XBP1 and ATF6 pathways exert the strongest influence on transcriptional remodeling (**Chapter I**). I have shown XBP-1s and ATF-6 are involved in *C. elegans* transgenic models of tauopathy (**Chapter II**). Both *xbp-1s* and *atf-6* loss of function enhanced tauopathy phenotypes. Using an overexpression model of the XBP-1s UPR^{ER} master transcription factor, I demonstrated XBP-1s expression drives tauopathy suppression (**Chapter II**). Furthermore, *atf-6* loss of function abolished the ability of *xbp-1s* to suppress tauopathy phenotypes (**Chapter II**).

These data suggest the possibility that ATF-6n transcriptional regulation may also suppress tauopathy phenotypes in *C. elegans*. Future studies are necessary to dissect the effect of ATF-6n function in *C. elegans* tauopathy models. To do this, generation of a *C. elegans* transgenic model overexpressing *atf-6n* in neurons is required to explore the consequences on tauopathy phenotypes. Because *xbp-1s* overexpression did not fully eliminate neuronal tau toxicity, future experiments could also explore the combinatorial effect of XBP-1s and ATF-6n function in tauopathy suppression by generating a *C. elegans* transgenic model overexpressing both transcription factors.

To understand the relationship of UPR^{ER} transcriptional activation in *C. elegans* transgenic models of tauopathy, I performed transcriptomic analysis to identify essential upregulated target genes mediated by XBP-1s (**Chapter III**). I further narrowed down the candidate genes to those with conservation in mammals and any known association with the ATF6 UPR^{ER} transcriptional branch and nominated five genes: *csp-1*, *dnj-28*, *hsp-4*, *ckb-2*, and *lip1-3* (**Chapter III**). I demonstrated the four genes *csp-1*, *dnj-28*, *hsp-4*, and *lip1-3* are all required for *xbp-1s*-mediated tauopathy suppression in *C. elegans* (**Chapter III**). Furthermore, HSP-4 function modulated tauopathy phenotypes: *hsp-4* loss of function enhanced tauopathy behavioral phenotypes, and

hsp-4 transgenic overexpression mildly suppressed tauopathy behavioral phenotypes which was dependent on expression level (**Chapter III**). Future studies should investigate the role of the other four nominated target genes (*csp-1*, *dnj-28*, *ckb-2*, and *lip1-3*) in *C. elegans* tauopathy, specifically whether neuronal overexpression can ameliorate tauopathy phenotypes. This work is necessary to determine whether UPR^{ER} transcriptional reprogramming requires coordinated action of multiple target genes to ameliorate tauopathy in *C. elegans*.

I chose to conduct *C. elegans* whole organism isolation of RNA at the L2 larval stage for enrichment of neuronal transcripts (**Chapter III**). The tau and *xbp-1s* transgenic *C. elegans* models I used are pan-neuronally driven and constitutively expressed throughout the lifespan. However, this method excludes the potential contribution of XBP-1s transcriptional remodeling in adulthood. Previous studies in *C. elegans* demonstrate both biological responses and gene expression differ significantly in different stages of development^{282,283}. Understanding adult cell-specific transcriptional changes in *C. elegans* is important for comparison of gene expression changes in other adult organisms such as humans. Because tauopathy is associated with aging in humans, future work should explore XBP-1s-mediated transcriptional reprogramming in adulthood in tau transgenic *C. elegans*. A previous study demonstrated successful isolation of GFP-labeled neurons in day 1 adult *C. elegans* via fluorescence-activated cell sorting (FACS) and subsequent RNA sequencing analysis of neuron-specific transcripts²⁸⁴. Using this technique, future experiments can isolate the neuronal contributions of XBP-1s in adulthood in tau transgenic *C. elegans* and compare these upregulated transcripts to the transcriptional results in whole organism L2 larval stage animals. Additionally, this method can be used to explore transcriptional changes in *xbp-1s*-mediated tauopathy suppression with *atf-6* loss of function to identify unique upregulated genes dependent on ATF-6 function. If a successful *atf-6n* neuronal overexpression transgenic model is made and able to modulate tauopathy phenotypes, neuronal-specific transcriptomic analysis can also be conducted to fully understand the independent contribution of

the UPR^{ER} XBP-1s and ATF-6n transcription factors in *C. elegans* transgenic models of tauopathy.

Mechanisms of XBP-1s-mediated tauopathy suppression in *C. elegans*

The UPR^{ER} stress response functions to restore ER proteostasis defects by promoting overall cellular function and survival via transcriptional and translational remodeling which affects multiple subcellular compartments (**Chapter I**). After confirming tau protein does not reside in the ER compartment in tau transgenic *C. elegans*, I investigated how constitutive activation of the XBP-1s transcription factor might ameliorate tauopathy (**Chapter II**). Removal of aberrant proteins in the ER requires ERAD, which retrotranslocates proteins to the cytoplasm for subsequent degradation (**Chapter I**). I showed loss of function of SEL-11, an essential protein required for ERAD, is necessary for *xbp-1s*-mediated tauopathy behavioral suppression (**Chapter II**). Target genes of the UPR^{ER} XBP-1s and ATF-6n transcription factors include those involved in ERAD (**Chapter I**). The transcriptomic analysis of upregulated XBP-1s genes with a known association with ATF6 indicated four genes (*csp-1*, *dnj-28*, *hsp-4*, and *lip1-3*) are required for XBP-1s to suppress tauopathy in *C. elegans* (**Chapter III**). Given the subcellular compartment residency of these four proteins and their relationship to overall cellular viability, it is reasonable to further speculate ERAD activity may play a critical role in *xbp-1s*-mediated tauopathy suppression in *C. elegans*. However, future studies need to dissect the precise mechanism(s) of XBP-1s-mediated tau turnover in tau transgenic *C. elegans*, including monitoring activation of downstream cytoplasmic protein degradation target systems of ERAD such as autophagy and the UPS.

To investigate the role of XBP-1s in tauopathy suppression in *C. elegans*, I focused on the contribution of neuronal overexpression (**Chapter II, III**). The tau and *xbp-1s* transgenic *C. elegans* models I used specifically drive expression in neurons. However, initial investigation of

xbp-1s transgenic overexpression in *C. elegans* revealed tissue-specific contributions and non-cell autonomous effects in stress resistance and longevity¹⁹⁹. Future work should investigate whether XBP-1s activation in other cellular tissues such as the intestine and muscle¹⁹⁹ can also ameliorate tauopathy suppression. Although my results indicate neuronal overexpression of *xbp-1s* suppresses tauopathy phenotypes (**Chapter II**), it is necessary to explore whether whole organismal signaling effects of XBP-1s activation also contribute to neuronally-driven tauopathy in *C. elegans*.

Aging is a driver of proteostasis dysfunction such as the UPR^{ER} and neurodegenerative diseases, providing an intersection for exploring common underlying biological mechanisms of these diseases (**Chapter I**). Future work should focus on whether XBP-1s activation can also ameliorate pathological phenotypes in other human neurodegenerative disease transgenic *C. elegans* models. A variety of *C. elegans* neurodegenerative disease models of ALS, tauopathies, PD, and HD exist¹²⁷. In our laboratory, we have utilized a transgenic polyQ model that has a pan-neuronal expression of an 86 amino acid glutamine tract^{285,286}. Our laboratory has generated TDP-43 proteinopathy models by pan-neuronally expressing human wildtype and ALS-causing mutant TDP-43²⁸¹. Specific to AD, our laboratory has generated transgenic *C. elegans* models exploring the relationship between tau and other pathological proteins associated with disease. Our laboratory previously developed a robust model of AD by pan-neuronally expressing both human A β ₁₋₄₂ and wildtype tau proteins²⁸⁷. Our laboratory also generated the first model demonstrating synergistic proteotoxic effects of pan-neuronal expression of wildtype tau and TDP-43²⁸⁸. To understand whether XBP-1s overexpression can modulate other pathological proteins, it is necessary to explore the effects of *xbp-1s* neuronal overexpression in other neurodegenerative proteinopathy transgenic *C. elegans* models. These studies will provide insight regarding whether UPR^{ER} transcriptional activation via XBP-1s is specific to tau proteotoxicity or generalizable to other aggregation-prone proteins in *C. elegans*.

Highly conserved UPR^{ER} nominates transcriptional activation may be involved in tauopathy suppression in mammals

The *C. elegans* model system provides many advantages for dissecting the underlying mechanisms of pathology in human neurodegenerative diseases that are not easily achieved in intact mammalian model systems (**Chapter I**). I have shown XBP-1s and ATF-6 UPR^{ER} transcriptional branches ameliorate tauopathy phenotypes (**Chapter II**) and identified essential conserved transcriptional target genes *csp-1*, *dnj-28*, *hsp-4*, and *lip1-3* (**Chapter III**) in human tauopathy transgenic *C. elegans* models. However, to understand how these results impact human tauopathies, future studies should employ mammalian model systems. Our laboratory utilizes two main human tau transgenic mouse models which express different tau species in the CNS and exhibit a range of tauopathy phenotypes. Previously, our laboratory generated the transgenic 2652 tauopathy mouse model, which expresses high levels of wildtype tau in neurons and drives pre-tangle neuropathology in the absence of neurofibrillary tangle formation, resulting in behavioral abnormalities with no effect on lifespan²⁸⁹. In another laboratory, a transgene encoding human tau containing the P301S mutation found in human FTDP-17 driven by a neuronal-specific promoter was used to create PS19 transgenic mice²⁹⁰. In contrast to 2652 transgenic animals, PS19 animals develop neurofibrillary tangles, progressive behavioral defects, neurodegeneration, and reduced lifespan²⁹⁰.

Taken altogether, the work in this dissertation provides the basis for further investigation into the translational capability of the UPR^{ER} transcriptional branches and nominated upregulated target genes discovered in *C. elegans* to modify tauopathy phenotypes in mammalian systems, with the long-term goal of identifying novel therapeutic targets in human tauopathy.

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