

## Journal Club

**Editor's Note:** These short, critical reviews of recent papers in the *Journal*, written exclusively by graduate students or postdoctoral fellows, are intended to summarize the important findings of the paper and provide additional insight and commentary. For more information on the format and purpose of the Journal Club, please see [http://www.jneurosci.org/misc/ifa\\_features.shtml](http://www.jneurosci.org/misc/ifa_features.shtml).

## Tau Pathology as a Cause and Consequence of the UPR

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Review of Abisambra et al.

The endoplasmic reticulum (ER) is a site of protein processing and quality control in the cell, replete with mechanisms for recognizing misfolded proteins and resolving proteotoxic stress. These control mechanisms are collectively known as the ER unfolded protein response (UPR; reviewed by Schröder and Kaufman, 2005). UPR signal transduction pathways are orchestrated by the coordinated actions of three key transmembrane proteins: inositol-requiring enzyme 1 $\alpha$  (IRE1 $\alpha$ ), activating transcription factor 6 (ATF6), and protein kinase RNA-like ER kinase (PERK). Together with the ER chaperone BiP, which helps ensure proper protein folding, these factors mediate a rapid and multifaceted response to the accumulation of unfolded proteins in the ER lumen. When activated, the UPR initially induces selective suppression of protein translation, and elevates the expression of chaperones and antioxidant proteins. Additionally, the UPR promotes the stepwise removal of persistently misfolded proteins through ER-associated degradation (ERAD) and the ubiquitin proteasome system (Schröder and Kaufman, 2005). However, chronic activation of the UPR

elicits a transition from these generalized prosurvival responses toward one of cell death via apoptosis. Neurological disorders such as Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis (ALS), and Alzheimer's disease (AD) feature both UPR activation and ERAD dysfunction (Roussel et al., 2013).

In AD and other neurodegenerative diseases collectively called tauopathies, the microtubule-associated protein tau becomes hyper-phosphorylated and forms insoluble intracellular aggregates associated with disease progression (reviewed by Spillantini and Goedert, 2013). Although a tremendous amount of research has investigated the role of tau in neurodegeneration, the underlying mechanisms of tau toxicity remain elusive.

In their recent article in *The Journal of Neuroscience*, Abisambra et al. (2013) offer insight into the nature of tau toxicity by revealing that aberrant soluble tau species impair ERAD and activate the UPR. The authors first demonstrate that the appearance of phosphorylated PERK (pPERK), an early marker of incipient ER stress and UPR activation, accompanies the accumulation of soluble tau in rTg4510 mice. These animals conditionally express P301L mutant human 4R0N tau, which has an increased tendency to aggregate and is associated with the heritable tauopathy, frontotemporal dementia (Alonso Adel et al., 2004; Santacruz et al., 2005). At 9 months of age, levels of pPERK, the ER chaperone BiP, and ubiquitinated proteins were higher in transgenic animals than in controls (Abisambra et al., 2013, their Fig. 1). Doxycycline administration suppressed the expression of soluble tau and

concomitantly reduced levels of pPERK (Abisambra et al., 2013, their Fig. 5D–H).

Next, Abisambra et al. (2013) induced expression of wild-type human 4R0N tau in HEK cells lacking detectable endogenous tau. Levels of pPERK increased in parallel with soluble transgenic tau, peaking ~72 h after induction (Abisambra et al., 2013, their Fig. 2A–B). Ubiquitinated proteins rapidly accumulated before PERK phosphorylation, and BiP continued to accumulate beyond 72 h. Cessation of tau overexpression significantly reduced pPERK levels. These data suggest that 4R0N tau expression at least transiently activates the UPR in HEK cells.

Elevated levels of ubiquitinated proteins accompanied tau accumulation in both HEK cells and rTg4510 animals, and this was also observed in the brains of AD patients (Abisambra et al., 2013, their Figs. 1A, 2A, 3, and 4). The majority of this ubiquitinated protein pool was not attributable to pathologically phosphorylated cytosolic tau. Notably, in rTg4510 mice and AD brains, 17 and 31% of respective ubiquitin immunostaining colocalized with the ER membrane protein calnexin, whereas immunostaining of phosphorylated tau did not (Abisambra et al. 2013, their Figs. 3–4). These observations raised the possibility that general proteasomal degradation and ERAD may be impaired in the context of both experimental murine tauopathy and human AD tau pathology.

Consistent with this possibility, tau was detected ectopically in microsomes isolated from tau-expressing HEK cells. In rTg4510 mice, tau coimmunoprecipitated

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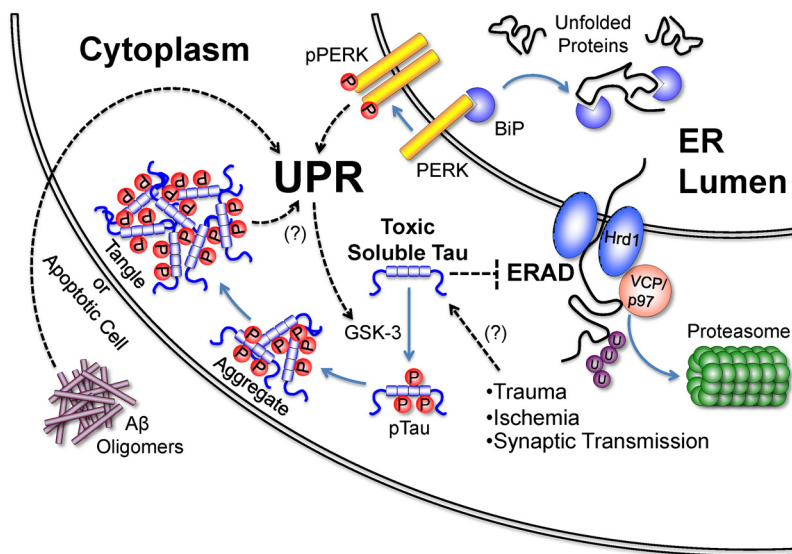
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with two cytosolic components of the ERAD and ubiquitin proteasome machinery: VCP/p97 and Hrd1 (Abisambra et al., 2013, their Fig. 6). CD3 $\delta$  (a protein normally degraded via ERAD) accumulated in microsomal fractions isolated from tau-expressing HEK cells and rTg4510 animals, indicating ERAD dysfunction. Strikingly, CD3 $\delta$  accumulation was also identified in tissue from AD patients (Abisambra et al. 2013, their Fig. 6K–L). These results implicate tau as a cause of ERAD dysfunction, and in light of previously observed UPR activity, suggest protein accumulation in the ER lumen arising from decreased efflux via ERAD.

The authors suggest that impaired ERAD and UPR activation leads to cell death, potentially directly linking tau and neurodegeneration. There are important caveats to this conclusion, however. First, as mentioned above, the UPR is thought to initially serve a prosurvival function, only transitioning to proapoptotic signaling upon chronic or severe ER stress. In this regard, Abisambra et al. (2013) do not show involvement of classically proapoptotic UPR factors such as TRAF2, CHOP, ATF4, or caspases 12, 9, and 3 (Schröder and Kaufman, 2005). Second, their data do not include an assessment of cell viability following UPR activation by tau. This is unfortunate because wild-type and P301L variants of 4R0N tau result in distinct neurological pathologies when overexpressed in the brains of mice: P301L 4R0N tau induces neuronal degeneration whereas wild-type 4R0N tau does not, despite impairing synaptic function (Hoover et al., 2010). It is noteworthy, therefore, that Abisambra and colleagues found that both P301L and wild-type 4R0N tau similarly activate the PERK arm of the UPR. Collectively, this suggests that the P301L mutation is not completely responsible for the tau-induced ER stress observed *in vivo*, and that UPR activation in these cases might be ancillary to or independent of overt degeneration.

While the impact of tau-mediated UPR activation on cell viability remains unresolved, the results presented by Abisambra et al. (2013) show that ERAD dysfunction can result from the accumulation of soluble 4R0N tau, and that similar ERAD impairment occurs in the brains of AD patients. Furthermore, the reversible impact of tau expression on UPR activation as shown by Abisambra et al. (2013) supports the growing view that soluble tau species actively mediate tau toxicity, in addition to (or perhaps despite)



**Figure 1.** A hypothetical model of tauopathy promoted by ER stress in response to multiple stimuli. Activation of the UPR may result from toxic soluble tau species impairing ERAD through interactions with Hrd1 and VCP/p97, or directly via amyloid- $\beta$  toxicity. Impaired ERAD increases unfolded protein levels in the ER lumen, triggering the UPR by competitively titrating BiP from PERK. Subsequent PERK dimerization and autophosphorylation initiates UPR signaling. The UPR may then direct the misprocessing of tau via selective GSK-3 activation, facilitating tau aggregation. Unresolved UPR activity may alternatively result in cell death via apoptosis. A $\beta$ : Amyloid- $\beta$ , UPR: Unfolded Protein Response, GSK-3: Glycogen Synthase kinase 3, p: Phosphate group, U: Ubiquitin.

insoluble tau aggregates (Santacruz et al., 2005).

The results of Abisambra and coworkers differ from those presented by Spataro and Robinson (2010), who showed a lack of UPR induction by wild-type and P301L tau in HEK293 cells. This may reflect several key differences between the two studies. First, Spataro and Robinson evaluated UPR activation 24 and 48 h after induction of tau expression. Abisambra et al. (2013) show mild, nonsignificant UPR activation at these time points in their cell model (Abisambra et al., 2013, their Fig. 1A–B). Second, Abisambra and colleagues identified UPR induction via post-translational modification of PERK itself. In contrast, Spataro and Robinson (2010) assessed IRE-1 $\alpha$  activity and phosphorylation of the PERK substrate eIF2 $\alpha$ . PERK activity can be inferred by the detection of phosphorylated eIF2 $\alpha$  (Schröder and Kaufman, 2005), but Spataro and Robinson show a lack of robust eIF2 $\alpha$  phosphorylation in response to tau expression. It is therefore unfortunate that Abisambra et al. (2013) do not provide evidence of PERK activity in the form of phosphorylated eIF2 $\alpha$  at any time point in their study, as their data primarily indicate UPR activation beyond the time points assessed by Spataro and Robinson (2010).

In their discussion, Abisambra and colleagues speculate that tau accumulation seeds the production of additional

toxic tau species. Nijholt et al. (2013) provide a potential mechanism by which this might occur. These authors showed that UPR stimulation (indicated by pPERK and downstream eIF2 $\alpha$  phosphorylation) selectively activates glycogen synthase kinase 3 (GSK-3). GSK-3 is a serine/threonine kinase that directly phosphorylates tau at epitopes commonly identified in tau aggregates. As mentioned above, hyperphosphorylated tau is characteristic of tau pathology and hyper-phosphorylation is suggested to promote tau aggregation (Alonso Adel et al., 2004; Jeganathan et al., 2008). The UPR may therefore initiate two key aspects of tau pathology by generating abnormally phosphorylated tau species and promoting tau aggregation. In support of this possibility, Ho et al. (2012) demonstrated tau phosphorylation following UPR activation in primary neuronal cultures from rats.

Notably, the aggregation of tau has been suggested to promote cell survival by sequestering toxic soluble tau species into less toxic insoluble aggregates, similar to mutant huntingtin protein (Arrasate et al., 2004; Santacruz et al., 2005; Spires-Jones et al., 2009). One may therefore synthesize the findings of Abisambra et al. (2013) and others as follows: First, tau accumulation activates the UPR by impairing ERAD and activating GSK-3. This leads to persistent hyper-phosphorylation of tau and enhanced aggregation poten-

tial. If this occurs, then the hallmark tau aggregates seen in AD and related tauopathies might protect cells by sequestering toxic tau and preventing sustained UPR activation and apoptosis.

A model incorporating the above features is illustrated in Figure 1. This model accommodates multiple initial insults to the CNS, and may help describe the long-term tau-related sequelae observed in chronic traumatic encephalopathy (Hawkins et al., 2013), following transient cerebral ischemia (Wen et al., 2004), or perhaps from amyloid  $\beta$  ( $A\beta$ )-induced ER stress in AD. Uptake or misprocessing of soluble tau following traumatic brain injury or ischemia could ostensibly lead to tauopathy by causing ER stress. Trans-synaptic propagation of tau pathology, which has been proposed to be mediated by neuronal activity (Pooler et al., 2013) might similarly activate the UPR and promote tau pathology in adjacent cells by transmitting toxic or misprocessed tau. Such stimuli may introduce a cycle of ER stress, altered tau processing, and concomitant aggregation.

The recent association of polymorphisms in the *EIF2AK3* locus (encoding PERK) with progressive supranuclear palsy (Hoglinger et al., 2011) has genetically linked the UPR to tauopathy. Abisambra et al. (2013) hypothesize that such genetic variations might alter the expression of PERK, though detailed investigation of haplotype groups carrying the risk alleles has uncovered variations within the coding sequence of PERK, instead suggesting altered function of the protein itself (Stutzbach et al., 2013).

These possibilities require additional investigation. While it is unlikely that UPR activation and ERAD dysfunction are the only factors involved, the publication by Abisambra et al. (2013) highlights the relevance of ER homeostasis and stress response mechanisms in tau pathology. Their work demonstrates that ERAD can be hampered by tau, revealing a straightforward mechanism by which tau impairs cellular function. More broadly, these findings contribute to an increasingly nu-

anced view of tau in neurodegenerative disorders; one in which the enigmatic protein might behave as both a potent initiator of ER stress, and perhaps a downstream target of it as well.

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