Neuron
Previews

CellPress

Tau heckles speckles: A pathogenic mechanism in tauopathy?

Erica L. Gorenberg[1] and James Shorter[1,*]
[1]Department of Biochemistry and Biophysics, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA 19104, USA
*Correspondence: jshorter@pennmedicine.upenn.edu
https://doi.org/10.1016/j.neuron.2021.04.022

Protein aggregates can sequester RNA in neurodegenerative disease, but the exact RNAs sequestered by tau inclusions have remained uncharacterized. In this issue of *Neuron*, Lester et al. (2021) begin to identify these RNAs and reveal related perturbations in nuclear speckles.

Fibrillar tau aggregates are characteristic of a variety of neurodegenerative diseases, collectively termed tauopathies. The propagation of self-templating tau conformers is a pathological event in various tauopathies including Alzheimer's disease and specific forms of frontotemporal dementia (FTD). Curiously, although tau is not a canonical RNA-binding protein, it has long been known to engage RNA, and tau inclusions can sequester RNA in tauopathies (Ginsberg et al., 1997). Indeed, RNA can promote tau phase separation and fibrillization *in vitro* (Dinkel et al., 2015; Zhang et al., 2017). However, it has remained unclear how the presence of RNA in tau aggregates might contribute to disease progression.

In this issue of *Neuron*, Lester et al. begin to unravel the role of tau-RNA interactions by pinpointing the exact RNAs sequestered by tau inclusions in models of tauopathy (Lester et al., 2021). First, HEK293 tau biosensor cells were employed, which express the 4R repeat domain of tau, with the FTD-linked P301S mutation tagged with CFP or YFP. Fluorescent tau aggregates assemble in this cell line in the nucleus and cytoplasm upon transduction with non-fluorescent tau aggregates isolated from the brains of transgenic mice that express tau^{P301S}. These aggregated structures did not recover after photobleaching, indicating an immobile solid phase. Fluorescence *in situ* hybridization (FISH) revealed that both nuclear and cytoplasmic tau aggregates were enriched for poly(A) RNA. Likewise, in the hindbrain of 6-month-old tau^{P301S} mice, tau formed nuclear and cytoplasmic aggregates that harbored poly(A) RNA. Thus, nuclear and cytoplasmic tau aggregates sequester RNA in multiple model systems (Lester et al., 2021).

Next, the identities of the RNAs present in tau aggregates were determined. Tau aggregates were purified from HEK293 biosensor cells, and bound RNA was sequenced. A diverse set of RNAs were enriched in tau aggregates, including small non-coding RNAs, such as small nucleolar RNAs (snoRNAs) and small nuclear RNAs (snRNAs), as well as some tRNAs (Lester et al., 2021). Tau aggregates were also enriched for mRNAs that encode histones, centrosomal proteins, and splicing regulators. To validate these findings, tau aggregates were also purified from transgenic mouse brains that expressed FTD-linked tau^{P301L}. These tau aggregates were enriched for snoRNAs, similar to tau aggregates from HEK293 tau biosensor cells. However, rather than a general enrichment, tau aggregates from tau^{P301L} mouse brains were enriched for specific snoRNAs and showed only minor enrichment for snRNAs. Collectively, these findings establish that tau aggregates sequester specific RNAs and begin to reveal how tau aggregation might dysregulate the cellular RNA landscape (Lester et al., 2021).

Both nuclear and cytoplasmic tau aggregates were enriched for snRNAs and snoRNAs, but one of the highest enrichments was observed for U2 snRNA in nuclear tau aggregates (Lester et al., 2021). Typically, U2 snRNA is localized to nuclear speckles, which are RNA-protein condensates that promote gene expression and harbor RNAs and proteins involved in transcription, splicing, polyadenylation, RNA modification, and RNA export (Alexander et al., 2021; Galganski et al., 2017). Indeed, nuclear tau aggregates did not localize to the nucleolus but rather were colocalized with the nuclear-speckle protein SRRM2 in HEK293 tau^{P301S} biosensor cells, tau^{P301S}-expressing H4 neuronal cells, and transgenic tau^{P301S} mice. Strikingly, SRRM2 levels in the nucleus were reduced, and SRRM2 mislocalized to cytoplasmic tau aggregates in a manner that was dependent on the C-terminal, intrinsically disordered region of SRRM2. This change in SRRM2 localization did not affect nuclear-speckle integrity, as another speckle protein, SON, still localized to nuclear speckles. Several other nuclear-speckle components including PNN, SFPQ, and MSUT2 were also mislocalized to cytoplasmic tau inclusions, whereas others such as SON, SRSF1, SRSF2, and SRSF3 were not. These findings suggest that cytoplasmic tau aggregates might elicit changes in transcription and splicing driven by the depletion of nuclear SRRM2 and other select nuclear-speckle proteins (Lester et al., 2021).

What are the consequences of nuclear tau aggregation for the properties and functions of nuclear speckles? Fluorescence recovery after photobleaching (FRAP) studies revealed that tau aggregating in nuclear speckles reduced the mobility of the speckle residents SRRM2 and SRSF2, indicating that the material properties of nuclear speckles are altered by tau aggregates. Intriguingly, a change in the internal organization of nuclear speckles was also evident. In the absence of tau aggregates, SRRM2 and SON colocalized to the center of speckles. However, in the presence of tau aggregates, SRRM2 and tau aggregates colocalized to the center of nuclear speckles,

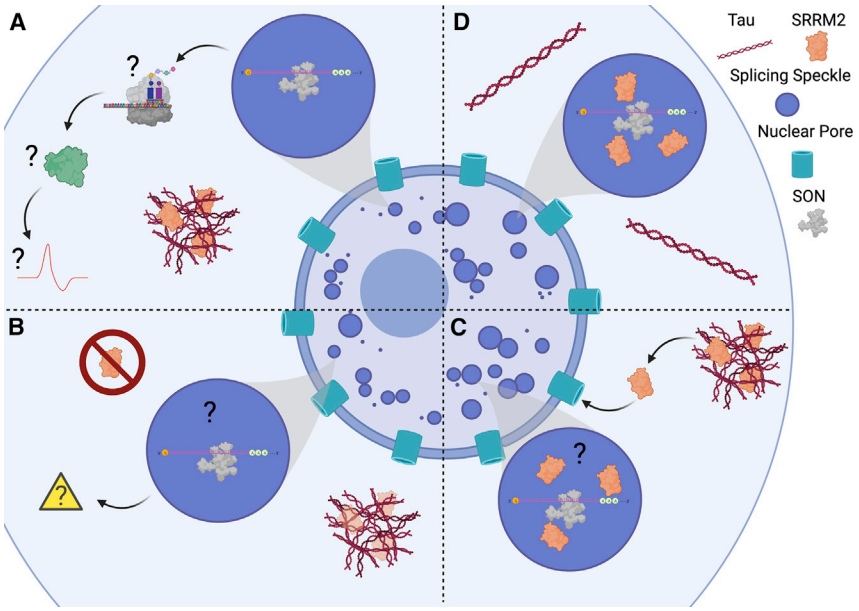

Figure 1. Overview of the effects of cytoplasmic tau aggregation on SRRM2 and possible therapeutic strategies

(A) Cytoplasmic tau aggregates sequester SRRM2 and deplete it from the nucleus. It will be interesting to determine how altered pre-mRNA splicing induced by SRRM2 mislocalization to cytoplasmic tau aggregates affects transcription, translation, and neuronal function.

(B) Altering SRRM2 expression levels could be explored to mitigate tau-aggregate-driven alterations in nuclear speckle function and RNA splicing.

(C) Exploiting nuclear-import receptors to extract SRRM2 from cytoplasmic tau aggregates and return it to the nucleus could help restore nuclear speckle function and mRNA splicing.

(D) Protein disaggregases could be exploited to solubilize tau aggregates and restore nuclear speckle homeostasis.

Created with BioRender.

whereas SON localized to the periphery (Lester et al., 2021). These findings suggest that nuclear tau aggregation disrupts the phase behavior and architecture of nuclear speckles.

Given these drastic alterations in speckle properties and nuclear depletion of SRRM2, the effects of tau aggregates on global pre-mRNA splicing were determined. RNA-seq on the HEK293 biosensor cells with or without tau aggregates revealed several striking shifts in splicing patterns. There were 305 splicing variations in 226 differentially spliced genes. Among the splicing variations, tau aggregates were associated with intron retention, alternative first exons, and alternative last exons. There were 1,225 introns retained in 641 genes in cells with tau aggregates as compared to 120 introns retained in 86 genes in the absence of tau aggregates. The pathways most impacted by splicing variations were the apoptosis and splicing associated protein (ASAP) complex, ribosome, and RNA splicing and processing. These findings raise the possibility of a self-reinforcing cycle of aberrant splicing elicited by tau aggregates (Lester et al., 2021). It will be important to determine whether these tau-aggregate-driven splicing changes affect specific neuronal genes that impact neurotransmission and drive neurodegeneration (Figure 1A).

Are these same effects of tau aggregates on SRRM2 also observed in mouse tauopathy models and human tauopathies? In wild-type mice, SRRM2 colocalized with poly(A) RNA to nuclear speckles, as expected. By contrast, in transgenic mice that expressed FTD-linked tau^{P301S}, SRRM2 mislocalized to cytoplasmic tau aggregates (Lester et al., 2021). Importantly, immunostaining of tauopathy patient brains revealed the presence of SRRM2 in cytoplasmic tau aggregates, whereas SRRM2 localized to nuclear speckles in healthy controls (Lester et al., 2021). Thus, it seems likely that the effects observed in cell models of tau aggregation are representative of human tauopathy.

In summary, Lester et al. establish that tau aggregates contain a range of specific RNAs. Nuclear tau aggregates induce alterations in nuclear speckles, and cytoplasmic tau aggregates sequester the nuclear-speckle component SRRM2, thereby depleting nuclear SRRM2 and eliciting widescale changes in pre-mRNA splicing (Lester et al., 2021). This study provides important insights into the role of RNA-tau interactions in tauopathy but leaves many questions open for further exploration.

First, can the tau-aggregate-mediated changes to RNA splicing be rescued by altering SRRM2 levels (Figure 1B)? Intriguingly, deletion of MSUT2, another nuclear-speckle resident that mislocalizes to cytoplasmic tau aggregates, mitigates neurodegeneration in transgenic mice that express FTD-linked tau^{P301S} (Wheeler et al., 2019). Thus, antisense oligonucleotides (ASOs) that reduce MSUT2 levels might represent a therapeutic opportunity for tauopathies. Might a similar approach mitigate SRRM2-mislocalization-induced changes as well?

Second, can SRRM2 splicing function be rescued by restoring SRRM2 nuclear localization in the presence of tau aggregates (Figure 1C)? Here, it may be interesting to explore upregulation of nuclear-import receptors, which can disaggregate their specific nuclear-localization signal (NLS)-bearing cargo in the cytoplasm and return it to the nucleus (Darling and Shorter, 2021). Importantly, transportin 3, the specific nuclear-import receptor for SRRM2, has recently been shown to possess chaperone and disaggregase activity (Baade et al., 2021). Thus, upregulation of transportin 3 might enable extraction of SRRM2 from cytoplasmic tau aggregates and restore SRRM2 to the nucleus. Likewise, other protein-disaggregase modalities such as Hsp104, VCP, TRIMs, or the Hsp70 chaperone system could be explored to directly solubilize aberrant states of tau and restore nuclear speckle homeostasis (Figure 1D) (Darling and Shorter, 2021).

Finally, is it possible to disrupt deleterious tau-RNA interactions using bait RNAs? Bait RNAs are short, specific RNA oligonucleotides (~20–40 nucleotides) that are designed to engage a

specific RNA-binding protein to prevent deleterious phase separation. For example, bait RNAs can effectively solubilize TDP-43 and counter neurodegeneration driven by cytoplasmic TDP-43 aggregation (Mann et al., 2019). Since bait RNAs are similar in size and character to ASOs, they could be readily delivered to degenerating neurons in patients. Clearly, further studies are urgently needed to understand how the exciting findings of Lester et al. might empower therapeutic strategies to mitigate neurodegeneration in tauopathies.

REFERENCES

Alexander, K.A., Cote, A., Nguyen, S.C., Zhang, L., Gholamalamdari, O., Agudelo-Garcia, P., Lin-Shiao, E., Tanim, K.M.A., Lim, J., Biddle, N., et al. (2021). p53 mediates target gene association with nuclear speckles for amplified RNA expression. Mol Cell *81*, 1666–1681.

Baade, I., Hutten, S., Sternburg, E.L., Pörschke, M., Hofweber, M., Dormann, D., and Kehlenbach, R.H. (2021). The RNA-binding protein FUS is chaperoned and imported into the nucleus by a network of import receptors. J. Biol. Chem. Published online April 12, 2021. https://doi.org/10.1016/j.jbc.2021.100659.

Darling, A.L., and Shorter, J. (2021). Combating deleterious phase transitions in neurodegenerative disease. Biochim Biophys Acta Mol Cell Res *1868*, 118984.

Dinkel, P.D., Holden, M.R., Matin, N., and Margittai, M. (2015). RNA Binds to Tau Fibrils and Sustains Template-Assisted Growth. Biochemistry *54*, 4731–4740.

Galganski, L., Urbanek, M.O., and Krzyzosiak, W.J. (2017). Nuclear speckles: molecular organization, biological function and role in disease. Nucleic Acids Res. *45*, 10350–10368.

Ginsberg, S.D., Crino, P.B., Lee, V.M., Eberwine, J.H., and Trojanowski, J.Q. (1997). Sequestration of RNA in Alzheimer's disease neurofibrillary tangles and senile plaques. Ann. Neurol. *41*, 200–209.

Lester, E., Ooi, F.K., Bakkar, N., Ayers, J., Woerman, A.L., Wheeler, J., Bowser, R., Carlson, G.A., Prusiner, S.B., and Parker, R. (2021). Tau aggregates are RNA-protein assemblies that mislocalize multiple nuclear speckle components. Neuron *109*, this issue, 1675–1691.e9.

Mann, J.R., Gleixner, A.M., Mauna, J.C., Gomes, E., DeChellis-Marks, M.R., Needham, P.G., Copley, K.E., Hurtle, B., Portz, B., Pyles, N.J., et al. (2019). RNA Binding Antagonizes Neurotoxic Phase Transitions of TDP-43. Neuron *102*, 321–338.

Wheeler, J.M., McMillan, P., Strovas, T.J., Liachko, N.F., Amlie-Wolf, A., Kow, R.L., Klein, R.L., Szot, P., Robinson, L., Guthrie, C., et al. (2019). Activity of the poly(A) binding protein MSUT2 determines susceptibility to pathological tau in the mammalian brain. Sci. Transl. Med. *11*, eaao6545.

Zhang, X., Lin, Y., Eschmann, N.A., Zhou, H., Rauch, J.N., Hernandez, I., Guzman, E., Kosik, K.S., and Han, S. (2017). RNA stores tau reversibly in complex coacervates. PLoS Biol. *15*, e2002183.

Basal ganglia reign through downstream control of motor centers in midbrain and brain stem while updating cortex with efference copy information

Sten Grillner[1,*] and William Scott Thompson[1]
[1]Department of Neuroscience, Karolinska Institutet, 17177 Stockholm, Sweden
*Correspondence: sten.grillner@ki.se
https://doi.org/10.1016/j.neuron.2021.04.015

In this issue of *Neuron*, McElvain et al. (2021) show that the major output of the basal ganglia, the substantia nigra pars reticulata, targets no fewer than 42 midbrain and brainstem structures and conveys an efference copy of the downstream commands back via thalamus to the cortex and striatum.

Much of the interest in the basal ganglia has been focused on the information fed back via thalamus to cortex, although it has long been known that there are important downstream connections to motor-related targets. The results that McElvain et al. (2021) report in this issue change, in a radical way, our understanding of the downstream control of movements and the type of information that is channeled back to cortex. The major output nucleus of the basal ganglia in rodents is the GABAergic substantia nigra pars reticulata (SNr). The authors show that neurons in the SNr target no fewer than 42 distinct regions, mostly in the midbrain and at the pontine and medullary brainstem level, consisting of, e.g., centers for the control of eye and orienting movements (Hikosaka and Wurtz 1983), posture, and locomotion, as well as orofacial structures. These projections are found to be highly segregated and support the notion of parallel channels of information processing through the basal ganglia.

The different SNr subpopulations are arranged in an orderly fashion from lateral to medial in the SNr (Figure 1). While all SNr neurons are spontaneously active, they exhibit heterogeneous physiological properties. McElvain et al. show that the lateral ones directed to the superior colliculus and the pontine and medullary reticular formation have a higher level of activity and briefer action potentials, whereas the medial ones projecting to, e.g., the raphe nuclei have longer-lasting action potentials and a lower rest rate. This topographic segregation within the SNr supports a model in which parallel modules exist throughout the basal ganglia (Figure 1; Grillner and Robertson 2016). Under resting conditions, SNr neurons continuously inhibit all their targets. An increased