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Transcellular Spreading of Tau in Tauopathies

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Tau, a microtubule-associated protein playing a key role in a group of neurodegenerative diseases such as Alzheimer's disease, spreads throughout the brain, inducing pathology. A model akin to the spreading of prions has been raised owing to similar characteristics of inducing an abnormal protein conformation as a method of self-amplification, spreading protein aggregates over anatomically linked pathways. The search to identify the "seeds" that induce conformational change has received much attention; however, less is known about the mechanisms by which tau is transmitted from cell to cell, so-called "transcellular spreading". In this review, we gather evidence regarding the spreading of tau throughout the brain and provide an overview of methods by which tau can be released from neurons as well as taken up. Furthermore, we bring together mechanisms of neurotoxicity behind tau spreading. Advancing our understanding about the spreading of tau can guide the search for therapeutic options for multiple neurodegenerative diseases aggregating tau.

1. Introduction

The protein tau plays a central role in a heterogeneous group of neurodegenerative diseases that are characterised by the accumulation of this protein. Called tauopathies, the most frequent is Alzheimer's disease (AD), which is immediately exceptional for the aggregation of a second protein, amyloid β (Aβ). The list of tauopathies also contains Pick disease, progressive supranuclear palsy (PSP), corticobasal degeneration (CBD), argyrophilic grain disease (AGD), Gerstmann–Straussler–Scheinker disease, British dementia, Danish dementia, Guam Parkinsonism–dementia complex, tangle-only dementia, white-matter tauopathy with globular glial inclusions, and frontotemporal dementia and Parkinsonism linked to chromosome 17 (FTDP).[1] These pathologies are clinically diverse, ranging from progressive gait instability and gaze palsy, which is suggestive of PSP, to the loss of memory, which is associated with AD.[2]

Tau is a microtubule-associated protein that is encoded by the *MAPT* gene on human chromosome 17q21.31; it plays a role in the assembly and stabilisation of microtubules, which is essential for axonal transport.[3] Traditionally considered an intracellular protein, tau is also secreted in the cerebrospinal fluid (CSF), with increased levels in AD, in which it is used for confirmation of the clinical diagnosis.[4] Hyperphosphorylation at the paired helical filament epitope 1 (PHF1) at Ser396 and Ser404, as in AD, causes a pathological change in conformation,[5] which leads to disassembly of the microtubuli and forms paired helical filaments (PHF) that aggregate further till neurofibrillary tangles.[6,7]

Braak and Braak postulated the concept that tau spreads throughout the brain on the basis of AD postmortem anatomopathological investigations related to progress in phenotype, classified into VI stages.[8] The hypothesis of spreading was further corroborated through the demonstration that small tau fibrils transmit to connected brain regions in transgenic mice, either wild-type tau-expressing animals after inoculation with brain extract from mutant P301S tau-expressing mice,[9] that is, mice overexpressing mutant human P301S tau after inoculation of synthetic preformed fibrils (pff),[10] or following injection of pff in mice expressing mutant human P301L tau.[11] Frost et al. showed earlier that extracellular tau aggregates enter cultured cells and trigger intracellular tau to form fibrils that are competent to seed further aggregation, referring to similarities with Prion propagation.[12] As such, the finding of tau spreading between connecting brain regions is backed up by mechanisms of transcellular spreading on the microscale. This model, called prion-like,[1,13,14] is defined as "the capacity of an assembled protein to induce the same abnormal conformation in a protein of the same kind, initiating a self-amplifying cascade".[15] The two main components of this propagation are, as such, conformational templating, exerted through "seeds" and transcellular spreading, implying spreading from one cell to another, to continue the definitions used by Lewis and Dickson.[16]

More arguments in favour of tau spreading have became available, including in vivo results that human P301L tau—known to cause FTDP[17]—propagates independent of mRNA expression, that is, no mRNA is detected in cells accumulating tau,[18] and more recently through the visualisation of tau on

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positron-emission tomography (PET) imaging.[19] The tau spreading hypothesis is, however, still not entirely accepted, and the "selective neuronal vulnerability" theory, which states that some cells are more vulnerable (e.g., genetic expression profiles) to the pathogenic processes initiating the disease, is an alternative explanation,[20] This is supported by the finding that neurons with long axons are especially affected.[21,22] Having high-energy needs makes these neurons more prone to oxidative stress, which establishes differential vulnerability.[22] However, as aforementioned, the affected neurons are anatomically connected, and therefore, the compatibility of both theories is not excluded.

In the adult human brain, as a result of alternative RNA splicing of exons 2, 3 and 10, six tau isoforms are expressed that range from 352 to 441 amino acids in length. Inclusion or not of the 31-amino-acid repeat sequence encoded by exon 10 of MAPT divides the tau isoforms into two categories, both of which are expressed in similar amounts in brain tissue with four (4R) or three microtubule-binding repeats (3R), respectively.[23] The 3R tau is physiologically the major foetal form, whereas the isoform with four tandem repeats is adult specific and also has higher microtubule polymerisation and binding capacity than 3R tau.[23,24] Neuronal inclusions in Pick's disease have 3R as the predominant tau isoform,[25] whereas filaments constituting mainly of 4R are indicative of PSP, CBD, or AGD.[26–28] Neuronal deposits in AD and tangle-only dementia (TD) contain both the 3R and 4R tau isoforms.[29,30]

The injection of brain homogenates of a number of human tauopathies in wild-type mice induces tau inclusions resembling the respective source pathology, thereby also showcasing tau propagation in rodents.[31] Similar experiments in transgenic mice inoculated with immunopurified tau from stable human tau-expressing cell clones from different tauopathies suggest that different diseases are associated with different sets of strains[32] described to spread differentially according to the disease of origin in experimental injections of transgenic mice with CBD or AD brain extracts enriched in pathological tau.[33] Differential spreading, as for AD classified by Braak and Braak[8] and for AGD classified by Saito et al.,[34] is in line with distinguished neuropathological features, such as in PSP atrophy of the subthalamic nucleus and brainstem tegmentum with depigmentation of the substantia nigra, whereas in CBD, asymmetric focal cortical atrophy with depigmentation of the substantia nigra is frequently found.[3] These tau strains, as known from Prion propagation theories, are a concept of unique "amyloid protein conformations". On the basis of biochemical and biological criteria, 18 different tau strains have been identified.[35] As such, the fibrillary structure dictates distinct cell pathology and progression rates and targets different brain regions, determining diverse pathological phenotypes.[35] The differing structures of tau filaments originating from the brains of AD[36] and Pick's disease patients[37] were recently demonstrated by cryoelectron microscopy, which revealed different folding as a result of residues K254–F378 of 3R tau in Pick's tau filaments that is likely to explain the selective incorporation of 3R tau in Pick bodies.[37] As such, conformers of assembled tau have been identified and have provided additional evidence for the claim that tau can have distinct folds in different diseases in the human brain.[37,38]

Unlike the conformations of tau and its mode of aggregation, the spreading mechanisms of tau are rather unacknowledged. Endorsing its crucial role in pathophysiology, an overview of the spreading of tau and the links inherently associated with the survival of tau and tau-mediated toxicity are provided in this review.

2. Synaptically Connected Spreading of Aggregates

The spreading of tau from the viewpoint of synaptic connectivity along with tau-induced synaptotoxicity is discussed first, and this is followed by examination of the mechanism of the transcellular spreading.

Since the first in vivo evidence of the trans-synaptic propagation of human wild-type and P301L tau was raised,[18,39,40] it has repeatedly been stated that synaptic connectivity, more than proximity, determines the pattern of spread.[41,42] Early evidence was based on the time-dependent propagation of phosphorylated tau to brain areas known to be anatomically interconnected.[11] More recently, experiments with microfluidic devices, which rule out all mechanisms of tau transfer besides synaptic transmission, have demonstrated the vital role of synaptic connectivity.[22] Notably, an alternative to trans-synaptic transmission, namely, microglia-mediated tau propagation, has also been suggested[43] (see below).

Tau transfers over synapses, and additional evidence suggests that tau exerts a possible pathologic role at pre- and postsynaptic terminals, which leads to the actual neurodegeneration. Postmortem analysis in AD patients has shown the presence and symmetrical distribution of misfolded tau over pre- and postsynapses.[44] Recent findings demonstrate the ability of human P301L tau to spread trans-synaptically before the degeneration of axonal terminals rather than through leakage.[45] Furthermore, Yoshiyama et al. have reported hippocampal synapse loss and impaired synaptic function in addition to prominent microglial activation prior to tau aggregate formation.[46]

Tau has its function at axonal microtubuli, although control human brains also show the presence of tau in dendritic spines.[47] Tau from pathological brains differs; for example, in AD, tau becomes hyperphosphorylated and misfolded.[47] Various authors have shown in vitro as well as in vivo that tau entry happens at somatodendritic compartments as well as at the axonal terminals and moves both anterogradely and retrogradely between the axon and neuronal body[41,48] (Figure 1, number 7). Thus, tau transfers not only trans-synaptically but also transcellularly.[16] This retrograde transport of tau to the somatodendritic compartments depends on hyperphosphorylation,[49] which allows tau to bypass the microtubule-binding-based diffusion barrier at the axonal initial segment.[50] Besides, retrograde transportation has also been shown for tau aggregates internalised at axonal terminals.[48]

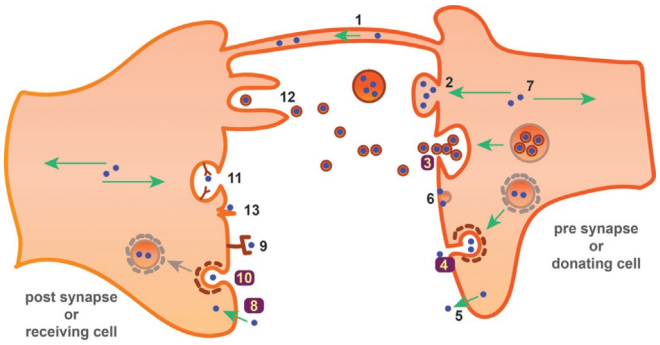

Figure 1. Schematic overview of the different spreading mechanisms of tau between two neurons. Only the numbers labelled in yellow with dark background are proven mechanisms for synaptic transmission; other mechanisms showcase transcellular spreading. Presynaptic: 1) tunnelling nanotube, 2) formation and budding off of ectosomes, 3) fusion of multivesicular body (MVB) with plasma membrane, releasing exosomes, 4) SNARE-based exocytosis, 5) tau release through plasma-membrane translocation, 6) presynaptic cytotoxic effects of tau: restriction of vesicle mobilisation, and 7) axonal antero- and retrograde transport of tau. Postsynaptic: 8) fluid-phase translocation, 9) postsynaptic cytotoxic effects of tau through binding of AMPA and NMDA receptors, 10) clathrin-mediated endocytosis, 11) HSPG-guided macropinocytosis, 12) exosome uptake with filopodia and 13) postsynaptic cytotoxic effects of tau through M1/M3 receptors, leading to calcium-ion influx.

2.1. Perisynaptic toxicity of tau

Postsynaptically, tau is essential for the dendritic localisation of Src kinase Fyn,[51] which in turn phosphorylates the *N*-methyl-D-aspartate (NMDA) receptor subunit 2 (NR2). Consecutively, NR2 interacts with the postsynaptic density protein 95 (PSD-95), which leads to synaptic excitotoxic downstream signalling.[51] This complex, crucial for mediating Aβ toxicity, bridges tau and Aβ neurotoxicity, and hence, dendritic targeting of Fyn is an attractive therapeutic approach for AD.[52] However, synaptic dysfunction does not only happen through pathways involving the NMDA receptor, as the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor is also involved in tau-related impaired glutamate receptor trafficking and synaptic anchoring[49] (Figure 1, number 9). In addition to neurotoxicity related to glutamate receptors, muscarinic receptors also play a role. Dephosphorylated tau is very stable extracellularly, and the affinity of dephosphorylated tau to bind the M1 and M3 muscarinic receptors is nearly tenfold higher than that of acetylcholine; moreover, unlike acetylcholine, dephosphorylated tau does not induce desensitisation upon repeated stimulation.[53,54] Through the aforementioned characteristics, dephosphorylated tau induces an increase in intracellular calcium, which leads to cellular toxicity as well as possible additional hyperphosphorylation and misfolding of tau[53–55] (Figure 1, number 13).

Presynaptically, tau has been shown to interfere with normal synaptic functioning through binding of synaptic vesicles by its N-terminal domain, while stimulating vesicle crosslinking by actin polymerisation. As such, vesicle mobilisation is restricted, which reduces the release rate and lowers neurotransmission[56] (Figure 1, number 6). Whereas normal tau binds to vesicles, both mutant FTDP-17 tau (R406W, V337M and P301L; 0N4R isoform) and hyperphosphorylated tau increase microtubular dissociation and enhance presynaptic localisation.[56]

3. Transcellular Spreading of Tau

Cell-to-cell spreading of tau can happen intercellularly through tunnelling nanotubes (TNTs)[57] or by release to extracellular space and subsequent cellular uptake.[58] The classification of structures differs from review to review, but here, a division into release of free extracellular tau versus vesicular transport seems logical (Figure 1). The first is theoretically possible through plasma-membrane translocation, secretory lysosomes and SNARE-mediated exocytosis; however, concrete evidence has been obtained only for the latter.[59] Regarding vesicular transport, a vast body of literature on exosomes as a secretion mechanism is available, and apart from that, ectosomes are also discussed.

The uptake of tau through fluid-phase endocytosis, particularly macropinocytosis, is discussed, and adsorptive endocytosis, an endocytotic process by which molecules are bound to the cell surface and concentrated before internalisation, with the molecules interacting preferentially with generic complementary binding sites, such as lectin or charged interaction, is noted.[60] Further, the different arguments in the clathrin-mediated endocytosis discussion are analysed and the uptake of intact exosomes is examined.

3.1. Intercellular spreading

TNTs, first described in 2004 by Rustom et al.,[61] are filamentous actin-containing channels that bridge remote cells with diameters of 50–200 nm and ship proteins to whole organelles de facto intercellularly from neuron to neuron. As such, TNTs act as ideal transfer agents, as proposed in prion proteins,[62] and have been suggested to be at least partially accountable for tau spreading (Figure 1, mechanism 1). Experiments by Tardivel et al. show that soluble tau is a constitutive component of TNTs, together with f-actin, and also demonstrate that fibrillar human tau 1N4R is transferred from neuron to neuron through TNTs.[57] It is so far unknown whether the detected tau fibrils are cytosolic tau or internalised in vesicles. Furthermore, exogenous tau fibrils favour the formation of TNTs. Another study confirms this and adds that tau fibrils are able to enter cells, which suggests self-enhancing propagation.[63]

3.2. Release

The finding of monomeric tau in the CSF of AD patients with early dementia onwards[64] leaves no doubt that tau is also present extracellularly, an essential first step in the cell-to-cell transmission of tau apart from the TNTs. In wild-type mice, monomeric tau has also been identified in the brain interstitial fluid (ISF), which supports physiological cellular release of tau.[65] Further experiments have confirmed active release of human tau in the absence of neuropathology[66] or cell death.[67] Finally, the identification of the exosomal transmission pathway has led Hall and Saman to the conclusion that tau is

secreted and that the extracellular appearance of tau is not a result of neuronal death.[68,69]

Continuing, neuronal activity stimulates release of largely dephosphorylated tau.[70] Pooler et al. have demonstrated calcium-dependent tau release in vitro in healthy cortical neurons upon AMPA receptor activation, but not upon NMDA receptor activation, on the basis of presynaptic exocytosis and largely non-exosomal. These findings of presynaptic glutamate-release-induced tau release are supported by using in vivo microdialysis in wild-type mice.[71] Potassium-induced depolarisation also induces release of both full-length and truncated tau from presynaptic terminals, and Sokolow et al. have shown that this release in postmortem AD synaptosomes is amplified relative to that in control tissue.[72]

3.3. Mechanisms of release

The first possible extracellular appearance of tau is as free tau, which has been shown for both truncated free tau[73] and full-length tau.[67] Immunoprecipitation of secreted, mainly truncated, dephosphorylated tau without any detergent also indicates that tau is not per se included in microvesicles or exosomes.[74] It is to be determined the extent to which extracellular free tau plays a physiological role on intracellular calcium through M1 and M3 muscarinic receptors[53] or is secreted because of intracellular abundance, which promotes pathological spreading. That assessment has therapeutic consequences in determining whether antibodies and anti-tau vaccine approaches, recently reviewed by Braczynski et al.,[75] should aim for intracellular tau or free extracellular tau.

Conventional protein secretion happens through vesicular incorporation, passing the endoplasmic reticulum (ER) and Golgi apparatus, and releasing the protein content in SNARE-mediated exocytosis.[59] Apart from that, there are unconventional pathways that consist of 1) direct plasma membrane crossing translocation from the cytoplasm, 2) release by secretory lysosomes, 3) microvesicle shedding and 4) exosome release.[76] Through the first two pathways, free protein is released, whereas through the latter two pathways, vesicles are released. A noncanonical pathway in which tau is driven out of the cell through SNARE-mediated exocytosis has been shown by Fontaine et al.[77] (Figure 1, mechanism 4). The mechanism starts with the constitutively expressed Heat shock cognate 70 (Hsc70), which in combination with DnaJ co-chaperones as a Hsc70–DnaJ complex and governs the triage of neurodegenerative proteins, including refolding, disaggregation and protein degradation.[77] The finding is that overexpression of DnaJC5, a DnaJ known to promote exocytosis, induces extracellular release of wild-type, P301L and R406W tau.[77] Thereby, synaptosomal-associated protein 23 (SNAP-23), a ubiquitously expressed SNARE protein involved in exocytosis, is engaged by DnaJC5.[77] As such, SNARE-mediated release of tau is linked with Hsc70–DnaJ-controlled proteostasis.

However, many authors make note of unconventional secretion processes that are independent of the ER/Golgi-mediated secretory pathway, as secretion can not be blocked by classic protein secretion inhibitors such as brefeldin A (BFA) and monensin[67] or by BFA-induced disassembly of the Golgi complex[66] and is strengthened by lack of tau in the synaptic vesicle proteome.[78] Besides, despite the fact that calcium homeostasis is critical in tau release, calcium is not required.[66] Further, whereas passive transport of proteins is unaffected by temperature change, heat-shock experiments in SH-SY5Y cells have revealed a significant increase in extracellular tau levels relative to cells incubated at 37 °C, which argues against passive diffusion.[66] Recently, this unconventional secretion mechanism has been elucidated to happen for full-length, soluble tau by direct plasma membrane translocation, which is favoured by tau hyperphosphorylation and mediated by sulfated proteoglycans[79] (Figure 1, mechanism 5).

Extracellular vesicles (EVs) can transmit tau pathology, and the presence of phosphorylated tau inside EVs has been evinced.[80] Besides, tau release has been linked to Rab GTPases, which play a role in regulating intracellular vesicle trafficking and membrane fusion. Given that RAB7A gene expression and protein levels are upregulated in AD patients, Rab7A deletion decreases tau secretion;[81] a similar effect is observed upon Rab1a suppression,[82] which further supports the release of tau through vesicle transport. Two common extracellular vesicles that are associated with tau, both detected in the medium of various cell lines under physiological conditions,[83] are ectosomes, also called microvesicles, which shed directly from the plasma membrane and are 100–1000 nm in diameter, and exosomes, which are 50–100 nm particles of endocytic origin[84] (Figure 1, mechanisms 2 and 3). Exosomes provide an acellular mode of communication, as they transfer molecules intercellularly[85] and trans-synaptically, and presynaptic release and postsynaptic uptake have been shown in *Drosophila* neuromuscular junctions.[86] Exosomes form by the internalisation and trafficking of endosomes to multivesicular bodies (MVBs), which upon fusion with the plasma membrane results in the release of exosomes.[87]

The secretion of exosomes by cultured cortical neurons[88] and by differentiated neurons has been demonstrated; it is regulated by calcium and glutamatergic activity.[89] In tau overexpression experiments, phosphorylated tau has been shown to be associated with exosomes,[68,90] on the basis of which Simón et al. have theorised that vesicular tau secretion is a cellular release mechanism so as to eliminate any excess amount of tau protein.[90] However, in experiments at physiological tau levels, tau has not been detected in the exosome fraction, which argues against vesicular tau release and points at tau leakage as a result of cell death as an explanation for the results obtained by both Saman et al. and Simón et al.[67] Furthermore, secreted exosomes contain no detectable tau in SH-SY5Y neuroblastoma cells, which express endogenous human tau,[66,91] and in primary cortical cultures.[88]

Contrary, a recent study provides extensive evidence for the secretion of both phosphorylated and dephosphorylated tau monomers and oligomers, as well as more complex aggregates, through exosomes by cultured neurons, N2a cells overexpressing human tau and in vivo in the human brain[22] (Figure 2). Neuronal activity stimulates exosomal tau secretion, with tau confirmed to be within the exosomes; the uptake of

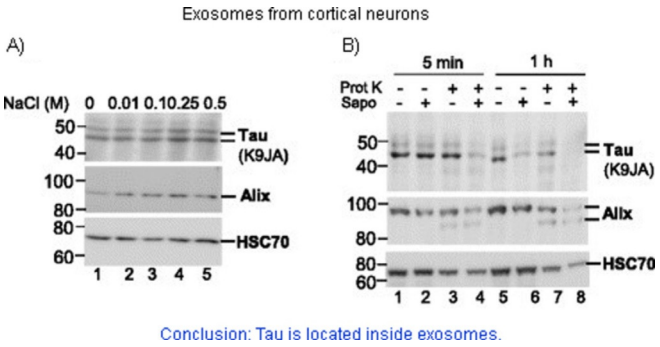

Figure 2. Tau is localised inside exosomes. A) Neuron-derived exosomes were incubated with increasing concentrations of NaCl to detach proteins peripherally attached to the membrane. Tau was detected with the pan-tau antibody K9JA. HSC70 and Alix were examined as exosomal markers. Lines on the right indicate tau protein, Alix and HSC70. M.W. markers are shown on the left. Note that exosomal tau levels are not changed by NaCl treatment (lanes 1–5), similar to exosomal markers HSC70 and Alix, which indicates that tau is not peripherally attached to the exosomal membrane surface. B) Proteinase K (Prot K) protection assay. Neuron-derived exosomes treated with or without 50 ng Prot K in the presence or absence of 1% saponin (Sapo) for 5 min or 1 h at 37 °C, followed by western blot analysis. Note that tau is strongly reduced (5 min) or even absent (1 h) in exosomes treated with both Prot K and Sapo compared with treatment with Prot K alone, which indicates that the exosomal membrane protects tau against Prot K digestion. Reproduced from ref. [116]. Copyright: 2017, Y. Wang et al.

tau-containing exosomes has been shown by neurons and microglia, not astrocytes.[22] Furthermore, these exosomal tau seeds are capable of inducing aggregation of aggregate-prone 4R tau with ΔK280 mutation after being taken up by recipient cells.[80]

Experiments with microfluidic devices have demonstrated not only that exosomes can conduct neuron-to-neuron tau spreading but, moreover, that synaptic connections are required for exosomal tau transmission.[22] However, in vivo microglia are also at play in tau transmission, as they efficiently phagocytise tau and secrete exosomal tau upon ATP stimulation to the extent that tau propagation is drastically reduced by microglial depletion or by pharmacologic inhibition of microglial exosome synthesis.[43] In vivo, tau P301L transduction to neurons is more efficient in microglia-derived exosomes than in free tau.[43] Besides, relatively more tau is insoluble in exosomes than in N2a cells; this suggests the preferential release of tau aggregates through exosomes. Combined with the fact that the seeding efficiency is higher in exosome-associated tau aggregates than in free tau aggregates from broken exosomes, these results stress the importance of the exosomal pathway in tau spreading.[22]

Tau is present in human CSF-derived exosomes[22] and in neuronally derived blood exosomes, which also show predictive value as biomarkers; the level of tau in human CSF-derived exosomes is significantly higher in AD patients compared to healthy control population.[92,93] These neuronal exosomes derived from the plasma of patients with mild cognitive impairment (MCI) converting into AD seed phospho-tau pathology in normal mice brains, contrary to MCI exosomes.[93] This blood-based biomarker could be, if further developed, a good replacement for the current existing CSF biomarkers (ratios of Ab42/total tau and Ab42/hyperphosphorylated tau), which require invasive sample collection.

As an explanation for the aforementioned results that refute exosomal tau, Wang et al. mention the need for the analysis of a sufficient amount of exosomes and the fact that the tau content is cell-type dependent and developmentally regulated.[22]

Apart from exosomes, ectosomes are also found in the medium of both primary neuronal cell cultures and cultured E17 rat cortical neurons.[83] Under physiological conditions controlled for cell damage, tau has been shown to be secreted through vesicular pathways other than exosomes, namely, ectosomes. Through NaCl washing, it has been confirmed that tau is inside the vesicles or anchored to the internal vesicle membrane. The release of this murine endogenous tau occurs for both the full-length form and the C- or N-truncated form. As such, these in vitro and in vivo experiments have shown that endogenous tau is released through ectosomes in the absence of cell damage. On the basis of the fact that a significantly higher amount of tau is released through ectosomes than through endosomes, until the point of overaccumulation, it is theorised that release happens preferentially through ectosomes and that overaccumulation of tau leads to the macroautophagic pathway of proteolysis, which can be described as the formation of multivesicular bodies (MVBs) that either target lysosomes or are secreted as exosomes.[83]

In a nutshell, tau transmission can take place trans-synaptically and transcellularly and either directly or with microglia as an intermediate step. Release happens as free tau through clathrin-mediated exocytosis as well as extracellular vesicles. These extracellular vesicles are defined as either exosomes or ectosomes.

3.4. Tau uptake

Similar to tau release, tau uptake can be divided into the uptake of free tau, either through passive transmembrane diffusion or active endocytosis, and the uptake of tau incorporated in vesicles. Kinetically, three forms of endocytosis processes for uptake of free tau can be distinguished, namely, fluid-phase endocytosis (bulk uptake of solutes), adsorptive endocytosis (molecules bind to cell surface and are concentrated before internalisation) and receptor-mediated endocytosis (specific ligand–receptor interaction).[60] However, there is parallel structuring based on morphology that partially overlaps, namely, macropinocytosis (nonspecific), caveolar endocytosis, clathrin-mediated endocytosis and clathrin-/caveole-independent endocytosis.[94]

3.4.1. Tau species

Before going into the uptake mechanisms, we first address the specifications of the tau species that are taken up. Compared with microtubule-binding region (MTBR) tau monomer [=amino acids 243–375 of full-length (P10636-8) wild-type tau], Frost et al. described significant more uptake of tau aggregates in C17.2 cultured cells.[12] In line with these results, it has been shown in human-induced pluripotent stem-cell

(hiPSC) experiments that tau oligomers based on recombinant human 2N4R tau, but not monomers, induce aggregation of pathological, hyperphosphorylated tau.[95] Upon measurement of recombinant and AD-derived tau aggregates based on tau repeat domain (RD; amino acids 243–375) and in cultured HEK293cells and primary cultured neurons, the minimum tau assembly has been shown to be a minimum of three units (trimers).[96] In a more recent study on a variety of cell models, including human CNS-derived cell lines and iPS-derived neurons, full-length human tau (2N4R) is also internalised efficiently, however, for both monomeric and oligomeric tau.[97] In another study, it was shown that tau fibrils could not be taken up, which suggests that there is a range of lengths of the tau species that are taken up by cells;[97] however, additional experiments are needed to elucidate the conflicting results regarding the uptake or not of tau monomers.

3.4.2. Mechanisms of uptake

Early evidence of tau aggregate uptake by cells pointed at fluid-phase endocytosis as the main mechanism of uptake[12,48,91] (Figure 1, mechanism 8), in addition to adsorptive endocytosis, as shown for misfolded preformed tau fibrils.[98] Co-localisation with dextran and temperature dependency indicate an active process of endocytosis rather than passive transmembrane diffusion, whereas caveolin-mediated endocytosis can be excluded.[48,99] Holmes et al. have specified this fluid-phase endocytosis as macropinocytosis, an actin-driven large invagination of the plasma membrane (0.5–10 μM) that internalises extracellular fluids[99] (Figure 1, mechanism 11). Extracellular tau RD fibrils stimulate their own internalisation by macropinocytosis, which is mediated in vitro and in vivo by heparan sulfate proteoglycans (HSPGs) on the cell surface.[99]

Significant clathrin-mediated endocytosis (CME) has been excluded by using Pit2B,[48] which is a small-molecule inhibitor of clathrin terminal domain function,[100] and by using siRNA knockdown of clathrin heavy chain (CHC),[99] as in both experiments tau aggregate uptake continues. Inhibition of dynamin, a guanosine triphosphatase involved in the fission reaction of synaptic vesicle endocytosis,[101] by using Dynasore (80 μM) has shown the endocytosis-dependent uptake of low-molecular-weight (LMW) tau species, which are oligomeric tau aggregates[48] (Figure 1, mechanism 10). This finding of the decreased propagation of tau pathology upon inhibited dynamin activity has been confirmed for TauP301L (1 μm Dynasore).[102] In a similar experiment (80 μM Dynasore), Holmes et al. show that tau internalisation is not reduced, which leads to the conclusion that tau RD fibril uptake is independent of dynamin 1.[99]

Regardless of the contradictory results on dynamin dependency, it has been shown that loss of Bin1, a protein that interacts with dynamin and negatively regulates endocytosis, promotes tau pathology.[102] This finding provides a pathophysiological mechanism for the fact that BIN1-amphiphysin2 is the second most prevalent genetic risk factor for late-onset Alzheimer's disease.[102]

In terms of vesicular mechanisms, exosomes are also recruited to the cell cytosol. This in an intact manner, surfing on filopodia, to be sorted into endosomal trafficking circuits[103] (Figure 1, mechanism 12). Exosomes might also make use of HSPGs for internalisation.[104]

In conclusion, cellular tau uptake happens through fluid-phase endocytosis, with macropinocytosis in particular, and through adsorptive endocytosis. Clathrin-mediated endocytosis is still under discussion, though the involvement of BIN1 is evident. Both plasma membrane translocation and caveolin-mediated endocytosis have been experimentally excluded. Furthermore, uptake of intact exosomes has been shown.

3.5. Intracellular sorting and survival

A crucial question in the whole tauopathy pathophysiology is what happens if tau becomes intracellular and what effect tau has on its host cell. Apart from the aforementioned synaptotoxicity, we elaborate on intracellular sorting, survival and tau-mediated neurotoxicity.

Internalisation does not only happen trans-synaptically, as Wu et al. have shown the endocytosis of full-length LMW tau aggregates in axonal and somatodendritic compartments, after which anterograde and retrograde trafficking takes place.[48] Tau aggregates, upon internalisation in endosomes, induce rupture of the vesicular membrane and, as such, provide a way for tau to escape to the cytoplasm, in which aggregate pathology can be further propagated.[102,105] For internalised exosomes containing tau, escape from the lysosomal pathway with consequential release has also been shown.[22] Similar to pore-like amyloid structures formed by amyloid β and α-synuclein, tau has been reported to have the capacity to form annular protofibrils (APFs) that disrupt both plasma and organelle-membrane pore-forming protein toxins, which are a means to calcium influx and neurotoxicity.[106] Tau oligomers, as a first step on the aggregation ladder, can either proceed towards neurofibrillary tangle formation through the formation of paired helical filaments (PHFs) that aggregate further or follow a distinct pathway to arrange themselves in APF structures that are found in the brain tissue of patients with PSP and dementia with Lewy bodies (DLB).[106]

Having tau floating in the cytosol, one might wonder why tau is not degraded through the ubiquitin-proteasome system (UPS) knowing that phosphorylated tau is polyubiquitylated by CHIP, an E3 ubiquitin ligase that interacts directly with Hsp70/90.[107,108] One answer lies in the acetylation of tau, which is one mechanism that prevents the ubiquitylation of phosphorylated tau and that is mediated through the histone acetyltransferase p300.[109] This acetylation of tau is increased in tauopathy patients through inhibition of p300 and, hence, reduces acetylation-enhanced tau degradation.[109] Besides acetylation, tau phosphorylation at specific KXGS residues (S262/S356 sites), which are within the MTBR,[110] also prevents CHIP ubiquitylation.[111] On the other hand, class III protein deacetylase SIRT1 is involved in deacetylation, but it is downregulated in AD patients and not in MCI subjects, and SIRT1 inversely correlates with the amount of total insoluble tau as well as with

other disease markers.[112] Furthermore, overexpression of the Hsp70 compound of the Hsp70/CHIP chaperone system reduces tau levels significantly in vivo, and this showcases the vital role of the selective elimination of abnormal tau species.[107]

With regard to the possible degradation escape pathways, a protein quality control (PQC) pathway, termed "misfolding-associated protein secretion" (MAPS), has recently been described, and it provides an explanation for the removal of defective proteins that have escaped degradation owing to proteasome insufficiency or dysfunction. Contrary to conventional PQC pathways that use degradation, MAPS is based on the secretion of misfolded proteins by using the ER-related deubiquitylase USP19. Although USP19 stimulates α-synuclein secretion, exosome-dependent tau secretion is not enhanced.[113]

4. Alternative Spreading Mechanisms

Apart from the foregoing mechanisms for transcellular spreading, we report a few other less straightforward mechanisms for tau spreading, and aside from the aforementioned microglial spreading, they presumably have limited roles in the pathogenesis of tauopathies.

In experiments involving the intraperitoneal injection of tau seeds resulting in intracerebral tauopathy, it has been revealed that peripheral tau, similar to prions and Aβ aggregates, can reach the central nervous system. As such, Clavaguera et al. have raised a hypothesis of blood cells as carrier of tau seeds, as is the case for amyloid seeds. However, it has to be noted that intraperitoneal administration of the brainstem extracts of homozygous P301S tau transgenic mice is less effective than intracerebral injection, and tauopathy only develops in heterozygous mice transgenic for human mutant P301S tau, not in wild-type mice.[114]

In light of research on cellular ageing, it has been found in *Escherichia coli* that dividing cells segregate protein aggregates asymmetrically.[115] Although not yet specifically reported for tau itself, this accumulation through asymmetric segregation is a theoretic possibility.

Microglia might play an essential role in tau spreading, as depletion of microglia suppresses tau propagation and reduces excitability in the dentate gyrus in a mouse model.[43] Furthermore, Asai et al. have raised the hypothesis that microglia might facilitate tau protein spreading between neurons by phagocytosing and exocytosing tau protein directly through exosomes or by phagocytosing neuronal axons containing tau to be secreted afterwards.

5. Conclusion

This review provides an overview of how tau spreads and which pathologic effects it exerts. The various spreading methods between neurons, either intracellularly, extracellularly or mediated through microglia, in addition to intracellular sorting, survival and neurotoxicity of tau on membranes and on pre- and postsynapse were discussed. Hence, we propose a model in which diverse spreading methods contribute to tau transmission and effect toxicity. Neuronal death might also elevate extracellular tau, which reinforces neurodegeneration as microglia try and fail in a cleaning operation. Neuronal release of tau seems to be a combination of physiological function and tau bypassing safety mechanisms, such as retrograde transport through phosphorylation.

The diversity of symptoms along the different neuropathies can be due to different propagation patterns throughout the brain. Upon looking back to the progress made in tauopathy research, we suggest that these macroscale variations are related to alternative transcellular spreading pathways, which leaves the option that each tauopathy has a disease-specific composition of multiple spreading methods. Furthermore, the toxic effects of tau might differ along the neuron population; for example, muscarinic receptor related neurotoxicity is likely more prevalent in cholinergic areas such as the entorhinal cortex. To complete the circle, the "selective neuronal vulnerability" theory could be the basis for the origin of pathologic tau seeds, which propagate throughout the brain along the prion-like model and exert their toxic effects on the involved neuronal networks, with all those complex interactions converging to a specific tauopathy. Although we mention "converging", we nevertheless leave the possibility that the tauopathies form a multidimensional spectrum rather than individual pathologic entities.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords: aggregation · cell-to-cell spreading · neurological agents · tauopathy · transmission

[1] M. Goedert, F. Clavaguera, M. Tolnay, *Trends Neurosci.* **2010**, *33*, 317–325.
[2] K. A. Josephs, *Mayo Clin. Proc.* **2017**, *92*, 1291–1303.
[3] G. G. Kovacs, *Neuropathol. Appl. Neurobiol.* **2015**, *41*, 3–23.
[4] S. Palmqvist, H. Zetterberg, N. Mattsson, P. Johansson, L. Minthon, K. Blennow, M. Olsson, O. Hansson, *Neurology* **2015**, *85*, 1240–1249.
[5] S. Jeganathan, A. Hascher, S. Chinnathambi, J. Biernat, E.-M. Mandelkow, E. Mandelkow, *J. Biol. Chem.* **2008**, *283*, 32066–32076.
[6] A. C. Alonso, I. Grundke-Iqbal, K. Iqbal, *Nat. Med.* **1996**, *2*, 783–787.
[7] E. M. Mandelkow, E. Mandelkow, *Cold Spring Harbor Perspect. Biol.* **2011**, *3*, 1–25.
[8] H. Braak, E. Braak, *Acta Neuropathol.* **1991**, *82*, 239–259.
[9] F. Clavaguera, T. Bolmont, R. A. Crowther, D. Abramowski, S. Frank, A. Probst, G. Fraser, A. K. Stalder, M. Beibel, M. Staufenbiel, M. Jucker, M. Goedert, M. Tolnay, *Nat. Cell Biol.* **2009**, *11*, 909–913.
[10] M. Iba, J. L. Guo, J. D. McBride, B. Zhang, J. Q. Trojanowski, V. M.-Y. Lee, *J. Neurosci.* **2013**, *33*, 1024–1037.
[11] E. Peeraer, A. Bottelbergs, K. Van Kolen, I. C. Stancu, B. Vasconcelos, M. Mahieu, H. Duytschaever, L. Ver Donck, A. Torremans, E. Sluydts, N. Va-

n Acker, J. A. Kemp, M. Mercken, K. R. Brunden, J. Q. Trojanowski, I. Dewachter, V. M. Y. Lee, D. Moechars, *Neurobiol. Dis.* **2015**, *73*, 83 – 95.
[12] B. Frost, R. L. Jacks, M. I. Diamond, *J. Biol. Chem.* **2009**, *284*, 12845 – 12852.
[13] P. Brundin, R. Melki, R. Kopito, *Nat. Rev. Mol. Cell Biol.* **2010**, *11*, 301 – 307.
[14] M. Jucker, L. C. Walker, *Nature* **2013**, *501*, 45 – 51.
[15] M. Goedert, M. G. Spillantini, *Mol. Brain* **2017**, *10*, 18.
[16] J. Lewis, D. W. Dickson, *Acta Neuropathol.* **2016**, *131*, 27 – 48.
[17] M. Hutton, C. L. Lendon, P. Rizzu, M. Baker, S. Froelich, H. Houlden, S. Pickering-Brown, S. Chakraverty, A. Isaacs, A. Grover et al., *Nature* **1998**, *393*, 702 – 705.
[18] A. De Calignon, M. Polydoro, M. Suarez-Calvet, C. William, D. H. Adamowicz, K. J. Kopeikina, R. Pitstick, N. Sahara, K. H. Ashe, G. A. Carlson, T. L. Spires-Jones, B. T. Hyman, *Neuron* **2012**, *73*, 685 – 697.
[19] M. Dani, D. J. Brooks, P. Edison, *Eur. J. Nucl. Med. Mol. Imaging* **2016**, *43*, 1139 – 1150.
[20] D. M. Walsh, D. J. Selkoe, *Nat. Rev. Neurosci.* **2016**, *17*, 251 – 260.
[21] H. Braak, K. Del Tredici, *Acta Neuropathol.* **2011**, *121*, 589 – 595.
[22] Y. Wang, V. Balaji, S. Kaniyappan, L. Krüger, S. Irsen, K. Tepper, R. Chandupatla, W. Maetzler, A. Schneider, E.-M. E. Mandelkow, E.-M. E. Mandelkow, *Mol. Neurodegener.* **2017**, *12*, 5.
[23] M. Goedert, M. G. Spillantini, R. Jakes, D. Rutherford, R. A. Crowther, *Neuron* **1989**, *3*, 519 – 526.
[24] M. Goedert, R. Jakes, *EMBO J.* **1990**, *9*, 4225 – 4230.
[25] A. Delacourte, Y. Robitaille, N. Sergeant, L. Buée, P. R. Hof, A. Wattez, A. Laroche-Cholette, J. Mathieu, P. Chagnon, D. Gauvreau, *J. Neuropathol. Exp. Neurol.* **1996**, *55*, 159 – 168.
[26] S. Flament, A. Delacourte, M. Verny, J. J. Hauw, F. Javoy-Agid, *Acta Neuropathol.* **1991**, *81*, 591 – 596.
[27] M. Tolnay, N. Sergeant, A. Ghestem, S. Chalbot, R. A. de Vos, E. N. Jansen Steur, A. Probst, A. Delacourte, *Acta Neuropathol.* **2002**, *104*, 425 – 434.
[28] H. Ksiezak-Reding, K. Morgan, L. A. Mattiace, P. Davies, W.-K. Liu, S.-H. Yen, K. Weidenheim, D. W. Dickson, *Am. J. Pathol.* **1994**, *145*, 1496 – 1508.
[29] M. Goedert, M. G. Spillantini, N. J. Cairns, R. A. Crowther, *Neuron* **1992**, *8*, 159 – 168.
[30] K. Noda, K. Sasaki, K. Fujimi, Y. Wakisaka, Y. Tanizaki, Y. Wakugawa, Y. Kiyohara, M. Iida, H. Aizawa, T. Iwaki, *Neuropathology* **2006**, *26*, 508 – 518.
[31] F. Clavaguera, H. Akatsu, G. Fraser, R. A. Crowther, S. Frank, J. Hench, A. Probst, D. T. Winkler, J. Reichwald, M. Staufenbiel, B. Ghetti, M. Goedert, M. Tolnay, *Proc. Natl. Acad. Sci. USA* **2013**, *110*, 9535 – 9540.
[32] D. W. Sanders, S. K. Kaufman, S. L. DeVos, A. M. Sharma, H. Mirbaha, A. Li, S. J. Barker, A. C. Foley, J. R. Thorpe, L. C. Serpell, T. M. Miller, L. T. Grinberg, W. W. Seeley, M. I. Diamond, *Neuron* **2014**, *82*, 1271 – 1288.
[33] S. Boluda, M. Iba, B. Zhang, K. M. Raible, V. M. Y. Lee, J. Q. Trojanowski, *Acta Neuropathol.* **2015**, *129*, 221 – 237.
[34] Y. Saito, N. N. Ruberu, M. Sawabe, T. Arai, N. Tanaka, Y. Kakuta, H. Yamanouchi, S. Murayama, *J. Neuropathol. Exp. Neurol.* **2004**, *63*, 911 – 918.
[35] S. K. Kaufman, D. W. Sanders, T. L. Thomas, A. J. Ruchinskas, J. Vaquer-Alicea, A. M. Sharma, T. M. Miller, M. I. Diamond, *Neuron* **2016**, *92*, 796 – 812.
[36] A. W. P. Fitzpatrick, B. Falcon, S. He, A. G. Murzin, G. Murshudov, H. J. Garringer, R. A. Crowther, B. Ghetti, M. Goedert, S. H. W. Scheres, *Nature* **2017**, *547*, 185 – 190.
[37] B. Falcon, W. Zhang, A. G. Murzin, G. Murshudov, H. J. Garringer, R. Vidal, R. A. Crowther, B. Ghetti, S. H. W. Scheres, M. Goedert, *Nature* **2018**, *561*, 137 – 140.
[38] M. Goedert, *FEBS Lett.* **2018**, *592*, 2383 – 2391.
[39] L. Liu, V. Drouet, J. W. Wu, M. P. Witter, S. A. Small, C. Clelland, K. Duff, *PLoS One* **2012**, *7*, e31302.
[40] S. Dujardin, K. Lecolle, R. Caillierez, S. Begard, N. Zommer, C. Lachaud, S. Carrier, N. Dufour, G. Auregan, J. Winderickx, P. Hantraye, N. Deglon, M. Colin, L. Buee, *Acta Neuropathol. Commun.* **2014**, *2*, 14.
[41] Z. Ahmed, J. Cooper, T. K. Murray, K. Garn, E. McNaughton, H. Clarke, S. Parhizkar, M. A. Ward, A. Cavallini, S. Jackson, S. Bose, F. Clavaguera, M. Tolnay, I. Lavenir, M. Goedert, M. L. Hutton, M. J. O'Neill, *Acta Neuropathol.* **2014**, *127*, 667 – 683.
[42] S. Calafate, A. Buist, K. Miskiewicz, V. Vijayan, G. Daneels, B. de Strooper, J. de Wit, P. Verstreken, D. Moechars, *Cell Rep.* **2015**, *11*, 1176 – 1183.
[43] H. Asai, S. Ikezu, S. Tsunoda, M. Medalla, J. Luebke, T. Haydar, B. Wolozin, O. Butovsky, S. Kügler, T. Ikezu, *Nat. Neurosci.* **2015**, *18*, 1584 – 1593.
[44] J. Avila, J. Lucas, M. Pérez, F. Hernández, *Physiol. Rev.* **2014**, *94*, 361 – 384.
[45] E. K. Pickett, C. M. Henstridge, E. Allison, R. Pitstick, A. Pooler, S. Wegmann, G. Carlson, B. T. Hyman, T. L. Spires-Jones, *Synapse* **2017**, *71*, 1 – 8.
[46] Y. Yoshiyama, M. Higuchi, B. Zhang, S. M. Huang, N. Iwata, T. C. Saido, J. Maeda, T. Suhara, J. Q. Trojanowski, V. M. Y. Lee, *Neuron* **2007**, *53*, 337 – 351.
[47] H. C. Tai, A. Serrano-Pozo, T. Hashimoto, M. P. Frosch, T. L. Spires-Jones, B. T. Hyman, *Am. J. Pathol.* **2012**, *181*, 1426 – 1435.
[48] J. W. Wu, M. Herman, L. Liu, S. Simoes, C. M. Acker, H. Figueroa, J. I. Steinberg, M. Margittai, R. Kayed, C. Zurzolo, G. Di Paolo, K. E. Duff, *J. Biol. Chem.* **2013**, *288*, 1856 – 1870.
[49] B. R. Hoover, M. N. Reed, J. Su, R. D. Penrod, L. A. Kotilinek, M. K. Grant, R. Pitstick, G. A. Carlson, L. M. Lanier, L. L. Yuan, K. H. Ashe, D. Liao, *Neuron* **2010**, *68*, 1067 – 1081.
[50] X. Li, Y. Kumar, H. Zempel, E.-M. Mandelkow, J. Biernat, E. Mandelkow, *EMBO J.* **2011**, *30*, 4825 – 4837.
[51] L. M. Ittner, Y. D. Ke, F. Delerue, M. Bi, A. Gladbach, J. van Eersel, H. Wölfing, B. C. Chieng, M. J. Christie, I. A. Napier, A. Eckert, M. Staufenbiel, E. Hardeman, J. Götz, *Cell* **2010**, *142*, 387 – 397.
[52] L. Rajendran, W. Annaert, *Traffic* **2012**, *13*, 759 – 770.
[53] A. Gómez-Ramos, M. Díaz-Hernández, A. Rubio, M. T. Miras-Portugal, J. Avila, *Mol. Cell. Neurosci.* **2008**, *37*, 673 – 681.
[54] A. Gómez-Ramos, M. Díaz-Hernández, A. Rubio, J. I. Díaz-Hernández, M. T. Miras-Portugal, J. Avila, *Eur. Neuropsychopharmacol.* **2009**, *19*, 708 – 717.
[55] S. S. Shafiei, M. J. Guerrero-Muñoz, D. L. Castillo-Carranza, *Front. Aging Neurosci.* **2017**, *9*, 83.
[56] L. Zhou, J. Mcinnes, K. Wierda, M. Holt, A. G. Herrmann, J. Rosemary, Y.-C. Wang, J. Swerts, J. Beyens, K. Miskiewicz, S. Vilain, *Nat. Commun.* **2017**, *8*, 1 – 29.
[57] M. Tardivel, S. Bégard, L. Bousset, S. Dujardin, A. Coens, R. Melki, L. Buée, M. Colin, *Acta Neuropathol. Commun.* **2016**, *4*, 117.
[58] J. W. Wu, S. A. Hussaini, I. M. Bastille, G. A. Rodriguez, A. Mrejeru, K. Rilett, D. W. Sanders, C. Cook, H. Fu, R. A. C. M. Boonen, M. Herman, E. Nahmani, S. Emrani, Y. H. Figueroa, M. I. Diamond, C. L. Clelland, S. Wray, K. E. Duff, *Nat. Neurosci.* **2016**, *19*, 1085 – 1092.
[59] C. Viotti, *Methods Mol. Biol.* **2016**, *1459*, 3 – 29.
[60] M. Amyere, M. Mettlen, P. Van Der Smissen, A. Platek, B. Payrastre, A. Veithen, P. J. Courtoy, *Int. J. Med. Microbiol.* **2001**, *291*, 487 – 494.
[61] A. Rustom, R. Saffrich, I. Markovic, P. Walther, H.-H. Gerdes, *Science* **2004**, *303*, 1007 – 1010.
[62] K. Gousset, E. Schiff, C. Langevin, Z. Marijanovic, A. Caputo, D. T. Browman, N. Chenouard, F. de Chaumont, A. Martino, J. Enninga, J.-C. Olivo-Marin, D. Männel, C. Zurzolo, *Nat. Cell Biol.* **2009**, *11*, 328 – 336.
[63] S. Abounit, J. W. Wu, G. S. Victoria, C. Zurzolo, *Prion* **2016**, *10*, 6896.
[64] K. Buch, M. Riemenschneider, P. Bartenstein, F. Willoch, U. Müller, M. Schmolke, T. Nolde, C. Steinmann, W. G. Guder, A. Kurz, *Nervenarzt* **1998**, *69*, 379 – 385.
[65] K. Yamada, J. R. Cirrito, F. R. Stewart, H. Jiang, M. B. Finn, B. B. Holmes, L. I. Binder, E.-M. Mandelkow, M. I. Diamond, V. M.-Y. Lee, D. M. Holtzman, *J. Neurosci.* **2011**, *31*, 13110 – 13117.
[66] C. M. Karch, A. T. Jeng, A. M. Goate, *J. Biol. Chem.* **2012**, *287*, 42751 – 42762.
[67] X. Chai, J. L. Dage, M. Citron, *Neurobiol. Dis.* **2012**, *48*, 356 – 366.
[68] S. Saman, W. H. Kim, M. Raya, Y. Visnick, S. Miro, S. Saman, B. Jackson, A. C. McKee, V. E. Alvarez, N. C. Y. Lee, G. F. Hall, *J. Biol. Chem.* **2012**, *287*, 3842 – 3849.
[69] G. F. Hall, S. Saman, *Commun. Integr. Biol.* **2012**, *5*, 623 – 626.
[70] A. M. Pooler, E. C. Phillips, D. H. W. Lau, W. Noble, D. P. Hanger, *EMBO Rep.* **2013**, *14*, 389 – 394.
[71] K. Yamada, J. K. Holth, F. Liao, F. R. Stewart, T. E. Mahan, H. Jiang, J. R. Cirrito, T. K. Patel, K. Hochgräfe, E.-M. Mandelkow, D. M. Holtzman, *J. Exp. Med.* **2014**, *211*, 387 – 393.

[72] S. Sokolow, K. M. Henkins, T. Bilousova, B. Gonzalez, H. V. Vinters, C. A. Miller, L. Cornwell, W. W. Poon, K. H. Gylys, *J. Neurochem.* **2015**, *133*, 368 – 379.
[73] N. Kfoury, B. B. Holmes, H. Jiang, D. M. Holtzman, M. I. Diamond, *J. Biol. Chem.* **2012**, *287*, 19440 – 19451.
[74] V. Plouffe, N.-V. Mohamed, J. Rivest-McGraw, J. Bertrand, M. Lauzon, N. Leclerc, *PLoS One* **2012**, *7*, e36873.
[75] A. K. Braczynski, J. B. Schulz, J.-P. Bach, *J. Neurochem.* **2017**, *143*, 467 – 488.
[76] W. Nickel, C. Rabouille, *Nat. Rev. Mol. Cell Biol.* **2009**, *10*, 148 – 155.
[77] S. N. Fontaine, D. Zheng, J. J. Sabbagh, M. D. Martin, D. Chaput, A. Darling, J. H. Trotter, A. R. Stothert, B. A. Nordhues, A. Lussier, J. Baker, L. Shelton, M. Kahn, L. J. Blair, S. M. Stevens, C. A. Dickey, *EMBO J.* **2016**, *35*, 1537 – 1549.
[78] S. Takamori, M. Holt, K. Stenius, E. A. Lemke, M. Grønborg, D. Riedel, H. Urlaub, S. Schenck, B. Brügger, P. Ringler, et al., *Cell* **2006**, *127*, 831 – 846.
[79] T. Katsinelos, M. Zeitler, E. Dimou, A. Karakatsani, H. M. Müller, E. Nachman, J. P. Steringer, C. Ruiz de Almodovar, W. Nickel, T. R. Jahn, *Cell Rep.* **2018**, *23*, 2039 – 2055.
[80] J. C. Polanco, B. J. Scicluna, A. F. Hill, J. Götz, *J. Biol. Chem.* **2016**, *291*, 12445 – 12466.
[81] L. Rodriguez, N. V. Mohamed, A. Desjardins, R. Lippé, E. A. Fon, N. Leclerc, *J. Neurochem.* **2017**, *141*, 592 – 605.
[82] N.-V. Mohamed, A. Desjardins, N. Leclerc, *PLoS One* **2017**, *12*, e0178288.
[83] S. Dujardin, S. Bégard, R. Caillierez, C. Lachaud, L. Delattre, S. Carrier, A. Loyens, M. C. Galas, L. Bousset, R. Melki, G. Aurégan, P. Hantraye, E. Brouillet, L. Buée, M. Colin, *PLoS One* **2014**, *9*, e100760.
[84] D. S. Choi, D. K. Kim, Y. K. Kim, Y. S. Gho, *Proteomics* **2013**, *13*, 1554 – 1571.
[85] B. Février, G. Raposo, *Curr. Opin. Cell Biol.* **2004**, *16*, 415 – 421.
[86] C. Korkut, Y. Li, K. Koles, C. Brewer, J. Ashley, M. Yoshihara, V. Budnik, *Neuron* **2013**, *77*, 1039 – 1046.
[87] M. Simons, G. Raposo, *Curr. Opin. Cell Biol.* **2009**, *21*, 575 – 581.
[88] J. Fauré, G. Lachenal, M. Court, J. Hirrlinger, C. Chatellard-Causse, B. Blot, J. Grange, G. Schoehn, Y. Goldberg, V. Boyer, F. Kirchhoff, G. Raposo, J. Garin, R. Sadoul, *Mol. Cell. Neurosci.* **2006**, *31*, 642 – 648.
[89] G. Lachenal, K. Pernet-Gallay, M. Chivet, F. J. Hemming, A. Belly, G. Bodon, B. Blot, G. Haase, Y. Goldberg, R. Sadoul, *Mol. Cell. Neurosci.* **2011**, *46*, 409 – 418.
[90] D. Simón, E. García-García, A. Gómez-Ramos, J. M. Falcón-Pérez, M. Daz-Hernández, F. Hernández, J. Avila, *Neurodegener. Dis.* **2012**, *10*, 73 – 75.
[91] I. Santa-Maria, M. Varghese, H. Ksiéżak-Reding, A. Dzhun, J. Wang, G. M. Pasinetti, *J. Biol. Chem.* **2012**, *287*, 20522 – 20533.
[92] M. S. Fiandaca, D. Kapogiannis, M. Mapstone, A. Boxer, E. Eitan, J. B. Schwartz, E. L. Abner, R. C. Petersen, H. J. Federoff, B. L. Miller, E. J. Goetzl, *Alzheimer's Dementia* **2015**, *11*, 600 – 607.e1.
[93] C. N. Winston, E. J. Goetzl, J. C. Akers, B. S. Carter, E. M. Rockenstein, D. Galasko, E. Masliah, R. A. Rissman, *Alzheimer's Dementia Diagn. Assess. Dis. Monit.* **2016**, *3*, 63 – 72.
[94] C. Lamaze, S. L. Schmid, *Curr. Opin. Cell Biol.* **1995**, *7*, 573 – 580.
[95] M. Usenovic, S. Niroomand, R. E. Drolet, L. Yao, R. C. Gaspar, N. G. Hatcher, J. Schachter, J. J. Renger, S. Parmentier-Batteur, *J. Neurosci.* **2015**, *35*, 14234 – 14250.
[96] H. Mirbaha, B. B. Holmes, D. W. Sanders, J. Bieschke, M. I. Diamond, *J. Biol. Chem.* **2015**, *290*, 14893 – 14903.
[97] J. N. Rauch, J. J. Chen, A. W. Sorum, G. M. Miller, T. Sharf, S. K. See, L. C. Hsieh-Wilson, M. Kampmann, K. S. Kosik, *Sci. Rep.* **2018**, *8*, 6382.
[98] J. L. Guo, V. M. Y. Lee, *J. Biol. Chem.* **2011**, *286*, 15317 – 15331.
[99] B. B. Holmes, S. L. DeVos, N. Kfoury, M. Li, R. Jacks, K. Yanamandra, M. O. Ouidja, F. M. Brodsky, J. Marasa, D. P. Bagchi, P. T. Kotzbauer, T. M. Miller, D. Papy-Garcia, M. I. Diamond, *Proc. Natl. Acad. Sci. USA* **2013**, *110*, E3138 – E3147.
[100] L. Von Kleist, W. Stahlschmidt, H. Bulut, K. Gromova, D. Puchkov, M. J. Robertson, K. A. Macgregor, N. Tomilin, A. Pechstein, N. Chau, M. Chircop, J. Sakoff, J. P. Von Kries, W. Saenger, H.-G. Krä, O. Shupliakov, P. J. Robinson, A. Mccluskey, V. Haucke, *Cell* **2011**, *146*, 471 – 484.
[101] S. M. Ferguson, G. Brasnjo, M. Hayashi, M. Wolfel, C. Collesi, S. Giovedi, A. Raimondi, L.-W. Gong, P. Ariel, S. Paradise, E. O'Toole, R. Flavell, O. Cremona, G. Miesenbock, T. A. Ryan, P. De Camilli, *Science* **2007**, *316*, 570 – 574.
[102] S. Calafate, W. Flavin, P. Verstreken, D. Moechars, *Cell Rep.* **2016**, *17*, 931 – 940.
[103] W. Heusermann, J. Hean, D. Trojer, E. Steib, S. von Bueren, A. Graff-Meyer, C. Genoud, K. Martin, N. Pizzato, J. Voshol, D. V. Morrissey, S. E. L. Andaloussi, M. J. Wood, N. C. Meisner-Kober, *J. Cell Biol.* **2016**, *213*, 173 – 184.
[104] H. C. Christianson, K. J. Svensson, T. H. van Kuppevelt, J.-P. Li, M. Belting, *Proc. Natl. Acad. Sci. USA* **2013**, *110*, 17380 – 17385.
[105] W. P. Flavin, L. Bousset, Z. C. Green, Y. Chu, S. Skarpathiotis, M. J. Chaney, J. H. Kordower, R. Melki, E. M. Campbell, *Acta Neuropathol.* **2017**, *134*, 629 – 653.
[106] C. A. Lasagna-Reeves, U. Sengupta, D. Castillo-Carranza, J. E. Gerson, M. Guerrero-Munoz, J. C. Troncoso, G. R. Jackson, R. Kayed, *Acta Neuropathol. Commun.* **2014**, *2*, 56.
[107] L. Petrucelli, D. Dickson, K. Kehoe, J. Taylor, H. Snyder, A. Grover, M. De Lucia, E. McGowan, J. Lewis, G. Prihar, J. Kim, W. H. Dillmann, S. E. Browne, A. Hall, R. Voellmy, Y. Tsuboi, T. M. Dawson, B. Wolozin, J. Hardy, et al., *Hum. Mol. Genet.* **2004**, *13*, 703 – 714.
[108] D. Cripps, S. N. Thomas, Y. Jeng, F. Yang, P. Davies, A. J. Yang, *J. Biol. Chem.* **2006**, *281*, 10825 – 10838.
[109] S. W. Min, S. H. Cho, Y. Zhou, S. Schroeder, V. Haroutunian, W. W. Seeley, E. J. Huang, Y. Shen, E. Masliah, C. Mukherjee, D. Meyers, P. A. Cole, M. Ott, L. Gan, *Neuron* **2010**, *67*, 953 – 966.
[110] C. A. Dickey, J. Dunmore, B. Lu, J.-W. Wang, W. C. Lee, A. Kamal, F. Burrows, C. Eckman, M. Hutton, L. Petrucelli, *FASEB J.* **2006**, *20*, 753 – 755.
[111] C. A. Dickey, A. Kamal, K. Lundgren, N. Klosak, R. M. Bailey, J. Dunmore, P. Ash, S. Shoraka, J. Zlatkovic, C. B. Eckman, C. Patterson, D. W. Dickson, N. S. Nahman, M. Hutton, F. Burrows, L. Petrucelli, *J. Clin. Invest.* **2007**, *117*, 648 – 658.
[112] C. Julien, C. Tremblay, V. Émond, M. Lebbadi, N. Salem, D. A. Bennett, F. Calon, *J. Neuropathol. Exp. Neurol.* **2009**, *68*, 48 – 58.
[113] J.-G. Lee, S. Takahama, G. Zhang, S. I. Tomarev, Y. Ye, *Nat. Cell Biol.* **2016**, *18*, 765 – 776.
[114] F. Clavaguera, J. Hench, I. Lavenir, G. Schweighauser, S. Frank, M. Goedert, M. Tolnay, *Acta Neuropathol.* **2014**, *127*, 299 – 301.
[115] A. B. Lindner, R. Madden, A. Demarez, E. J. Stewart, F. Taddei, *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 3076 – 3081.
[116] Y. Wang, V. Balaji, S. Kaniyappan, L. Krüger, S. Irsen, K. Tepper, R. R. Chandupatla, W. Maetzler, A. Schneider, E. Mandelkow, E.-M. Mandelkow, *Mol. Neurodegener.* **2017**, *12*, 5.

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