

## Review Article

# Tau Phosphorylation, Aggregation, and Cell Toxicity

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Protein aggregation takes place in many neurodegenerative disorders. However, there is a controversy about the possible toxicity of these protein aggregates. In this review, this controversy is discussed, focussing on the tau aggregation that takes place in those disorders known as tauopathies.

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## INTRODUCTION

Many neurodegenerative disorders are characterized by their presence in neural tissue of aberrant protein aggregates (see Table 1). In general, these aggregates arise after the modification of a native protein. That modification could result in a conformational change of the native protein that promotes the aberrant aggregation.

The most studied model of this mechanism has been the prion protein where a change from an alpha helix to beta sheet structure facilitates the polymerization of a protein with a different conformation that appears to have a cytotoxic effect [1].

In a similar way, conformational changes between a native protein and its aberrant protein counterpart with capacity for self-assembly have been studied in many neurodegenerative diseases. Among the most common techniques used for these analyses are X-ray diffraction, nuclear magnetic resonance, circular dichroism, or Fourier-transformed infrared spectroscopy. Similarly, to the case of prion protein, in some disorders a change from alpha helix to beta sheet conformation has been suggested to cause protein aggregation (Table 1), probably because in alpha helix, intramolecular hydrogen bonds could occur whereas intramolecular hydrogen bonds are facilitated in beta sheet conformation, facilitating protein aggregation. However, there is one case, the formation of aberrant aggregates of tau, where the aggregated protein contains also a high proportion of alpha helix structure [2].

Although, in some cases, like that of prion protein, the formation of aberrant aggregates of protein could result in a toxic effect in the affected neurons [1], in other cases, like

huntingtin aggregates, the formation of the aberrant aggregates could be a survival response of the affected neurons [3]. In other neurodegenerative diseases, it will be of interest to know if protein aggregation is synonymous of cell toxicity or not.

Protein conformation could also play a role in a possible toxic mechanism. In this way, a protein with a high proportion of alpha helix and hydrophobic regions could be inserted in cell membrane promoting toxic effects [4]. Additionally, the presence of aberrant polymers could affect the protein degradation cell machinery (the proteasome complex), decreasing its activity and promoting a toxic effect [5].

Recently, some good reviews have been published on protein aggregation and neurodegenerative disorders [6, 7]. In this review we will mainly focus on those aggregates assembled from tau protein, aggregates that could be present in the neurological disorders known as tauopathies (for a review see [8]) (Table 1).

## TAU AGGREGATION

It has been described that large amounts of native or unmodified tau protein were enough to promote tau assembly into fibrillar polymers resembling those found in AD [9–12]. Thus, obviously, an increase in tau concentration will favour the formation of tau polymers. Recently, it has been reported that not all the brain areas have a similar amount of tau protein [13]. Thus, it suggests a different probability in the formation of tau polymers in different brain regions.

The amount of tau will be the consequence of its synthesis and its degradation. Changes in transcription have

TABLE 1: Examples of neurological diseases where aberrant protein aggregates are found, and the suggested conformations in the aberrant aggregates are indicated.

Disease	Protein	Proteinopathies (aberrant aggregation)	
		Pathological finding	Protein conformation
Prion diseases	PrP <sup>Sc</sup>	PrP amyloid plaques	$\beta$ -sheet
	$A\beta$	$A\beta$ amyloid plaques	$\beta$ -sheet
Alzheimer's disease	Tau	Paired helical filaments in neurofibrillary tangles	$\beta$ -sheet + $\alpha$ -helix
	$\alpha$ -synuclein	Lewy bodies	$\beta$ -sheet + $\alpha$ -helix
Frontotemporal dementia	Tau	Straight filaments and paired helical filaments	—
Pick's disease	Tau	Pick bodies	—
Progressive supranuclear palsy	Tau	Straight filaments is neurofibrillary tangles	—
Amyotrophic lateral sclerosis	Neurofilament	Neural aggregates	—
Huntington's disease	Huntingtin	Nuclear inclusions	$\beta$ -sheet
Spinocerebellar ataxia			
Type 1	Ataxin 1	Nuclear inclusions	$\beta$ -sheet
Type 2	Ataxin 2	Cytoplasmic inclusions	$\beta$ -sheet
Machado-Joseph disease	Ataxin 3	Nuclear inclusions	$\beta$ -sheet

been indicated for other proteins related to neurodegenerative disorders [14], where a TATA binding protein may play a role. Tau degradation may take place through the proteasome complex [15, 16] and it has been suggested that such degradation could be regulated by posttranslational modifications occurring in tau molecule, like its phosphorylation [17]. Also, tau degradation by other proteases could be regulated by its level of phosphorylation [18]. It should be also indicated that in some cases like Parkinson's disease or Lafora disease, mutations in the E3 ubiquitin ligases like parkin [19] or malin [20] will result in the appearance of aberrant protein aggregates.

A conformational change, that could be followed by antibodies that react with tau molecule after that conformational change [21–24] has been also suggested to be required for the transition tau monomer-tau polymer.

Also, it has been suggested that different posttranslational modifications like phosphorylation [25], glycation [26], or truncation [27], may play a role in the formation of tau polymers.

Due to the alternative splicing of its heterogenous (or nuclear) RNA, different tau isoforms could be expressed and, therefore, different tau aggregates with different tau isoforms in different tauopathies could occur, but we will not discuss this point here. For further information see [8].

Mainly, studies on phosphorylation and truncation have been done. About truncation, it has been suggested that removal of the amino and/or carboxy terminal regions, leaving the tubulin binding region will facilitate tau polymerization [21, 22, 28].

Some work has been done in vitro [29] and in vivo [30, 31] about a possible role of tau phosphorylation on tau assembly, suggesting that in some conditions tau phosphorylation may increase the capacity of tau for its self-assembly. Not only an increase in serine/threonine phosphorylation of tau could regulate its aggregation but also an increase in tau tyrosine phosphorylation may increase the formation of tau aggregates [32]. This assembly process may involve the formation of oligomers [33], filaments, and aggregates of filaments (tangle-like structures). In the formation of these aggregates of filaments, glycation may play a role [26].

The possible relation between phosphorylation and tau aggregation has been studied in transgenic mice expressing human tau bearing some mutations found in human fronto-temporal dementia linked to chromosome 17 (FTDP-17) [31]. In this mouse, tau phosphorylation mainly occurs by GSK3 [34]. When a specific inhibitor of this kinase, lithium, was given to the transgenic mice no tau phosphorylation was found, and in addition no aggregation of the protein was detected [31] suggesting a correlation between tau phosphorylation and aggregation in this model. This result was supported by an additional experiment using another mouse also expressing human tau with a FTDP-17 mutation [30].

Alternatively, protein chaperones, acting on tau or in phosphotau, could modify the level of tau aggregation, examples could be the protein 14-3-3 [35], musashi-1 [36], or Pin-1 [37, 38]. The chaperone associated ubiquitin ligase CHIP could be able to target phosphotau for proteasomal degradation [16, 18].

## TOXICITY OF PHOSPHOTAU

It has been described that tau binding to microtubules is regulated by the level of tau phosphorylation at some specific sites [39]. It is known that hyperphosphorylated tau binds with less affinity to microtubules resulting in the decrease in the interaction with microtubules, in a decrease of microtubule stability [8], and probably in a microtubule dysfunction inside the cell that could result in a toxic effect [40]. Also, tau phosphorylation could result, as indicated above, in a decrease of its proteolysis [17]. More recently, it has been indicated that expression of a tau mutant (P301L) could result in an increase of its phosphorylation, since once it is phosphorylated, that mutation can prevent the binding of those phosphatases involved in its dephosphorylation [41]. This phosphotau could have a decreased capacity for microtubule binding and it could be toxic for the cell. Additionally, it has been indicated that hyperphosphorylated tau can cause neurodegeneration, in the absence of large tau aggregates [42]. On the other hand, only the overexpression of wild-type human tau in a mouse is sufficient to cause tau phosphorylation, aggregation, and neural toxicity [43]. On the other hand, it has been suggested that tau phosphorylation may represent a protective function in AD [44].

## TOXICITY OF AGGREGATED TAU

Sometime ago, the development of tau pathology, related with dementia in AD [45], was clearly described by Braak and Braak [46] by following the development of neurofibrillary lesions at different stages of the disease. Also, the formation of neurofibrillary (tau aggregates) pathology within those neurons of hippocampus and cerebral cortex affected at different stages was found. These neurons could degenerate yielding extracellular ghost tangles (eNFT) [47]. In the hippocampus, an inverse relation has been found between the number of eNFT and the number of surviving neurons [48–51]. It suggests that neurons that degenerate, have previously developed tau aggregates. On the other hand, it has been indicated that neurons bearing neurofibrillary lesions could survive for a long period of time [52], and, by comparing with other neurodegenerative disorders, like Huntington disease [3], it can be suggested that tau aggregates could protect against neurodegeneration by sequestering toxic (phospho?) monomeric tau that could be present in a high amount inside a cell in pathological conditions. Also, it has been suggested, using a transgenic mouse model [53], that behavioural (memory) deficits could be unrelated to the formation of tau polymers, although, more recently, the discussion of those experiments suggested that hyperphosphorylated, aggregated tau intermediates could be the ones that cause neurodegeneration [33]. In this way, the implication of different types of protein aggregates in neurodegeneration has been extensively discussed [19, 54]. A possibility about the existence of neurotoxic tau intermediate aggregates in human tauopathies is based in the fact that patients with FTDP-17 show an extensive neurodegeneration with a high level of tau phosphorylation but with a low number of tangles [55].

In any case, even if the formation of tau aggregates has a protective function for the neurons, that function is not working well, as described by Braak and Braak [46], and afterwards by Delacourte et al [56], indicating a correlation between progression of tau pathology and progression of the disease. This idea is supported by those experiments indicating that neural loss and neurofibrillary tangle number increase in parallel with the progression of the disease [57]. Similar results have been described in other neurological disorders like brain encephalopathies, where the formation of aberrant polymers are related to the onset of neurodegeneration [1]; whereas this is far from clear in other disorders like Huntington disease.

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