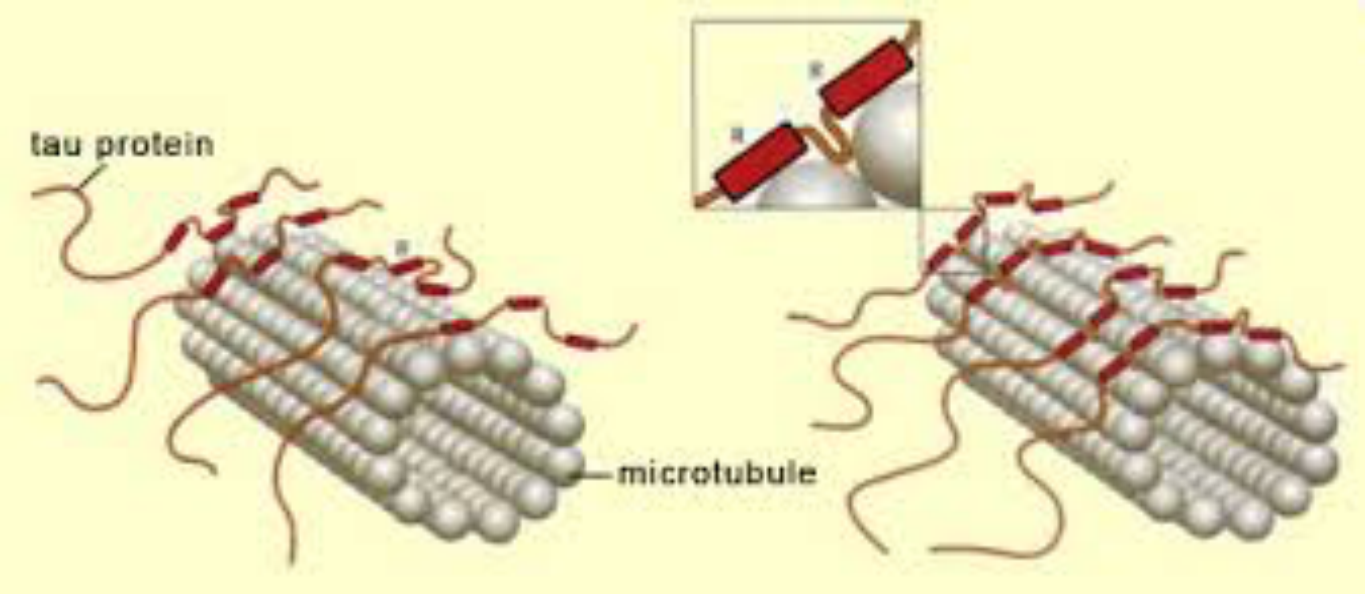
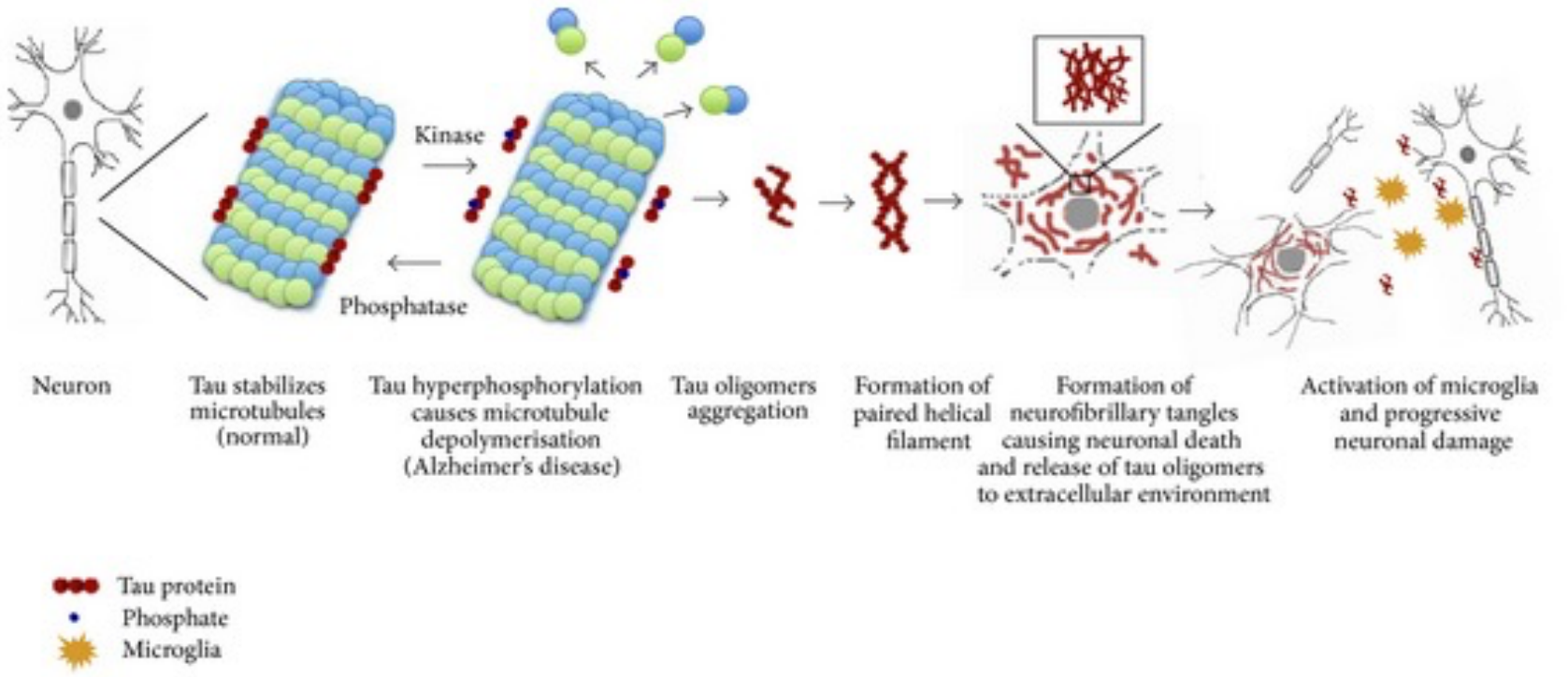
Structure and Pathology of Tau Protein in Alzheimer Disease

Tau protein was discovered in the mid-1970s of the 20th century by studying **factors necessary for microtubule formation**. Tau protein promotes tubulin assembly into microtubules, one of the major components of the neuronal cytoskeleton that defines the normal morphology and provides structural support to the neurons



Tubulin binding of tau is regulated by its phosphorylation state, which is regulated normally by coordinated action of kinases and phosphatases on tau Molecule. In pathological conditions, such as the case in AD, not only does abnormal phosphorylation of tau protein decrease its tubulin binding capacity leading to microtubule disorganization, but also this protein self-polymerizes and aggregates

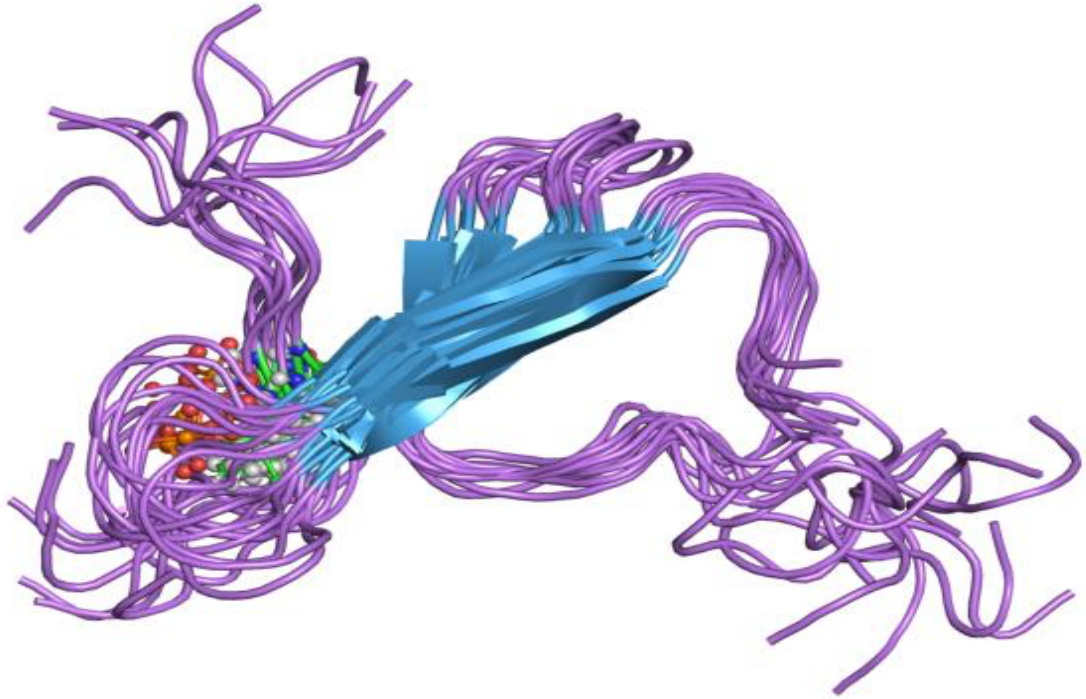


Structure and Function of Tau Protein

Tau protein belongs to a group of proteins referred to as Microtubule-Associated Proteins (**MAPs**), that in common are heat resistant and limited affected by acid treatment without loss their function. This property observed in tau is due to a very low content of secondary structure. In fact, a number of biophysical studies revealed that tau is a prototypical "**natively unfolded**" protein.

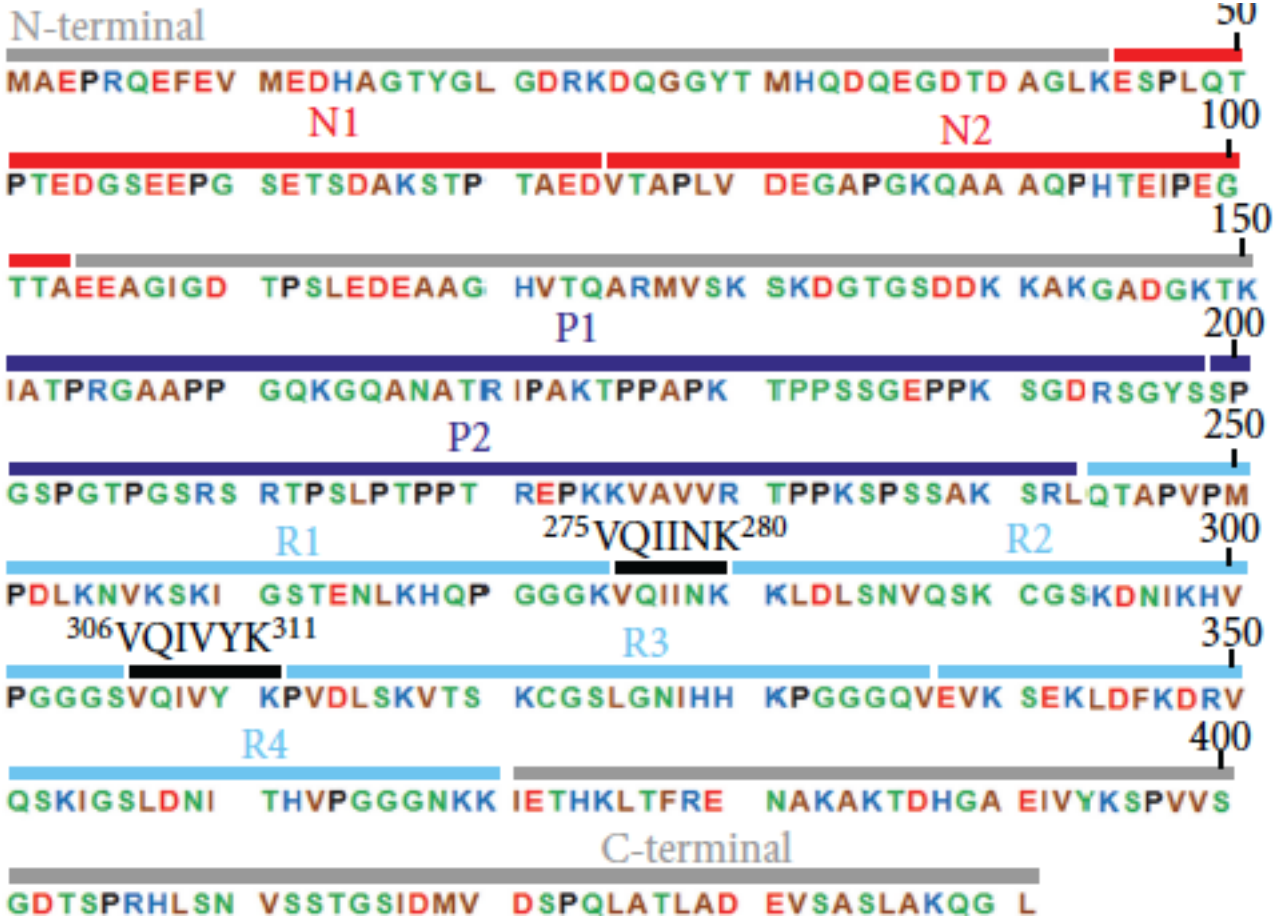
tau protein is a '*dipole*' with two domains of opposite charge, which can be modulated by posttranslational modifications .

Since disordered proteins tend to be highly flexible and have variable conformations, they have not been solved by crystallography so far. Thus NMR spectroscopy is the only plausible method that allows a description of their conformations and dynamics with high resolution.



Because of its hydrophilic nature Tau does not adopt a compact folded structure; in fact evidence suggests that the entire Tau molecule is "natively unfolded" or "intrinsically disordered". The polypeptide chain is highly flexible and mobile, there is only a low content of secondary structures, which are, however, transient. This corresponds to the observation that Tau can fulfill its physiological function of stabilizing microtubules even after harsh treatment (heat, acid,...).

Six isoforms of tau protein differ according to the contents of three (3R) or four (4R) tubulin binding domains (repeats, R) of 31 or 32 amino acids in the C-terminal part of tau protein and one (1N), two (2N), or no inserts of 29 amino acids each in the N-terminal portion of the molecule.

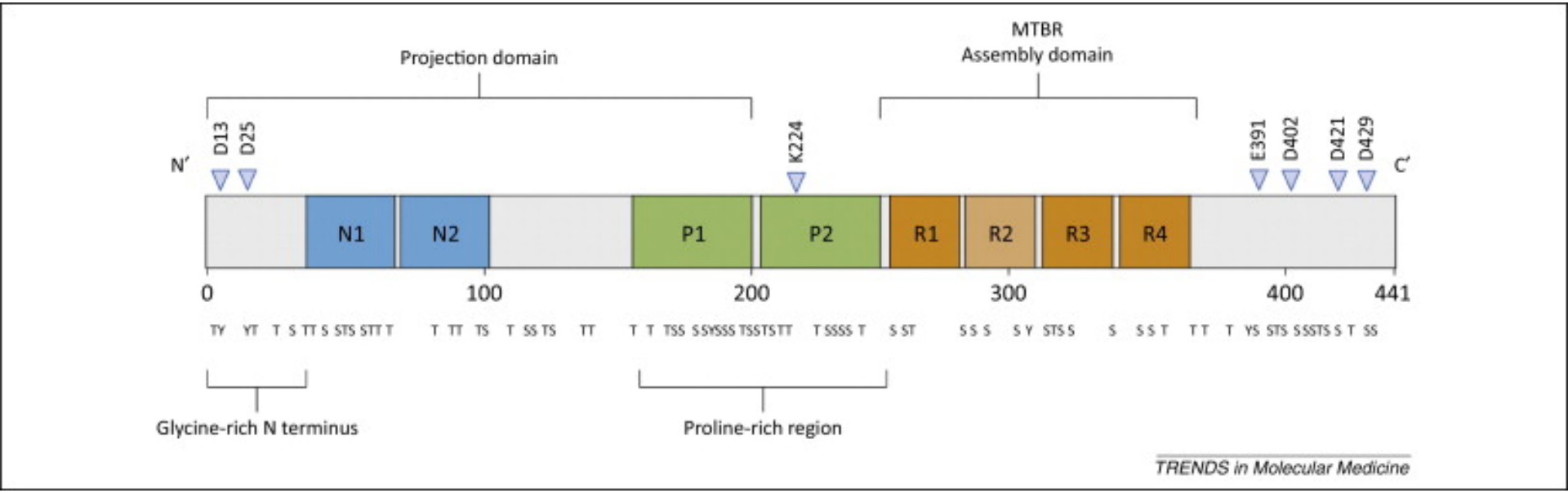


R1–R4: microtubule-binding domains

X = Basic AA (+)
X = Polar uncharged AA (hydrophilic)
X = Nonpolar AA (hydrophobic)
X = Acidic AA (−)

275VQIINK280 and 306VQIVYK311: sequences with βstructure

Amino acid sequence of the longest tau isoform (441 amino acids).



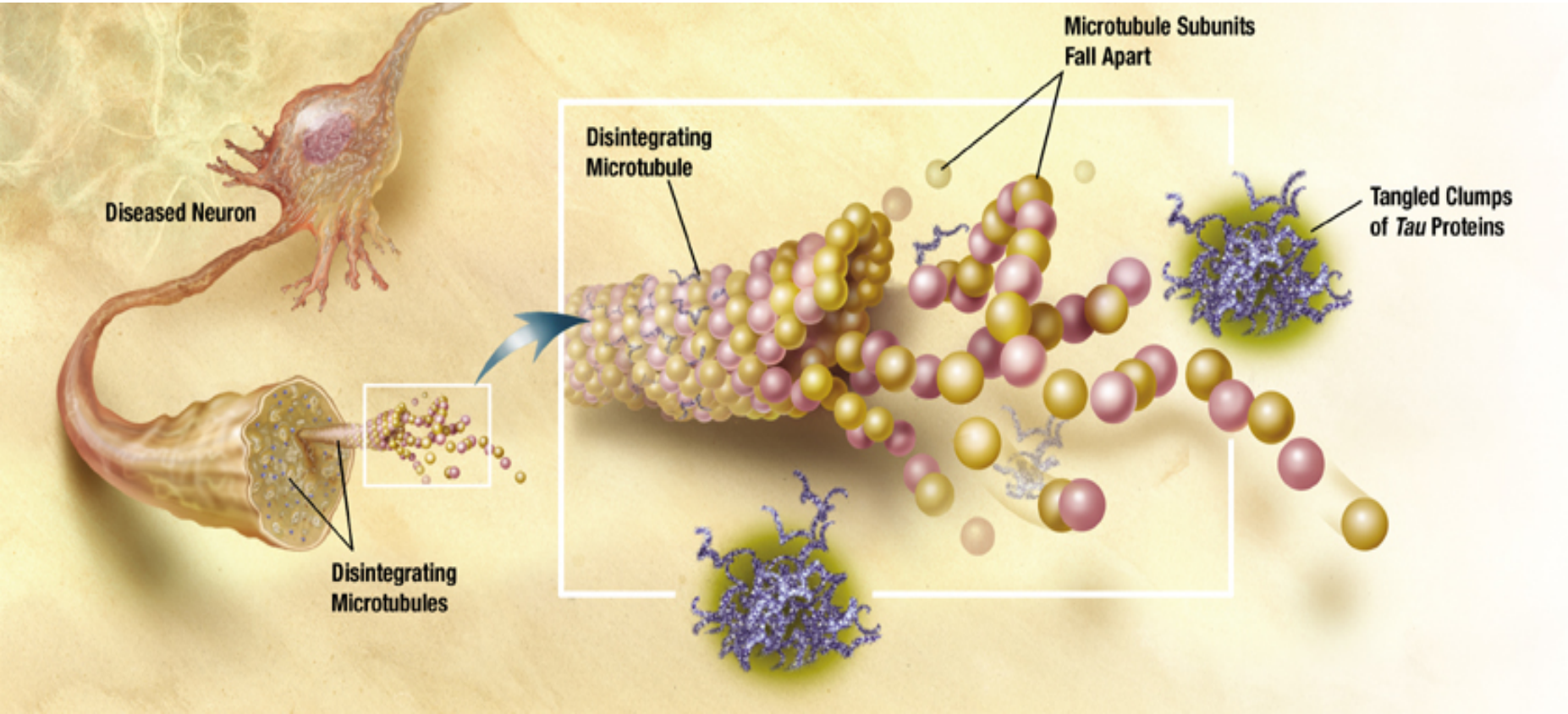
The six isoforms differ in the number of repeats at the C-terminal half and the number of inserts at the N-terminal half. The number of repeats may be either 3 or 4 and the number of inserts may be 0,1 or 2.

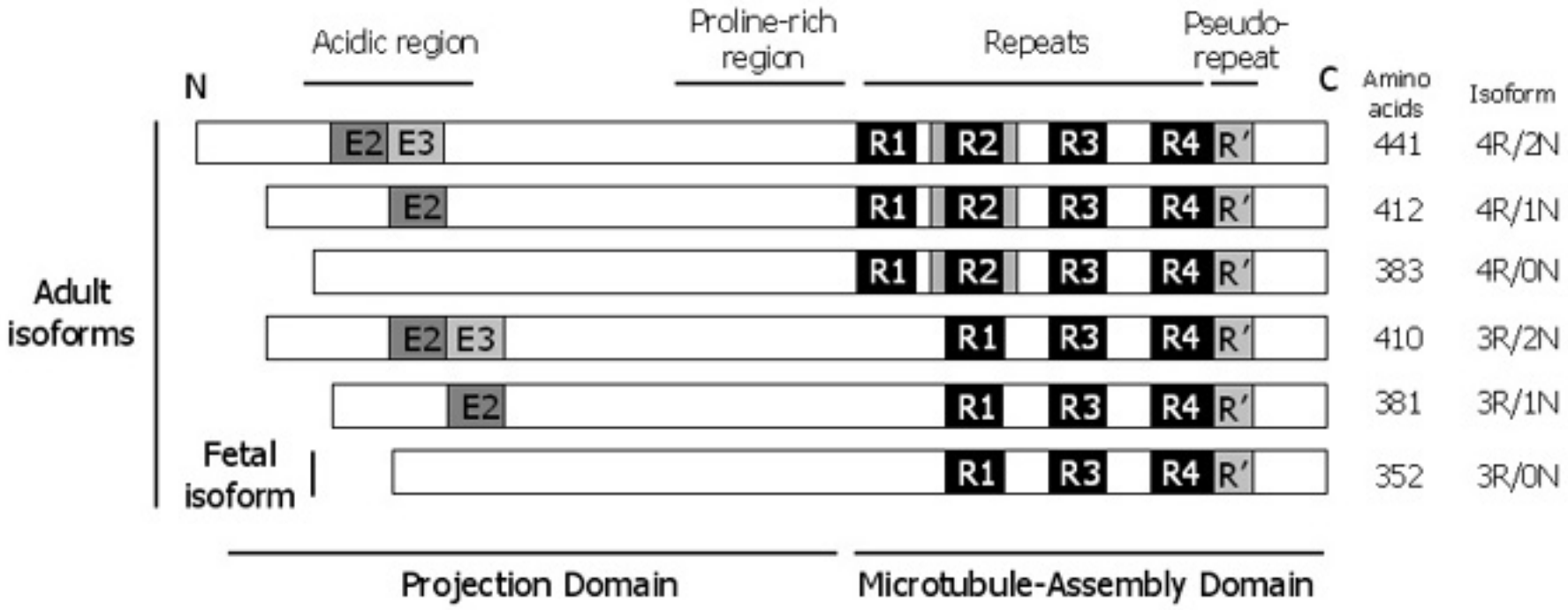
These six isoforms are also referred to as **τ3L, τ3S, τ3, τ4L, τ4S, and τ4**

Because each of these isoforms has specific physiological roles, they are differentially expressed during the development of the brain.

Tau proteins achieve their control of microtubule stability through **isoforms and phosphorylation**.

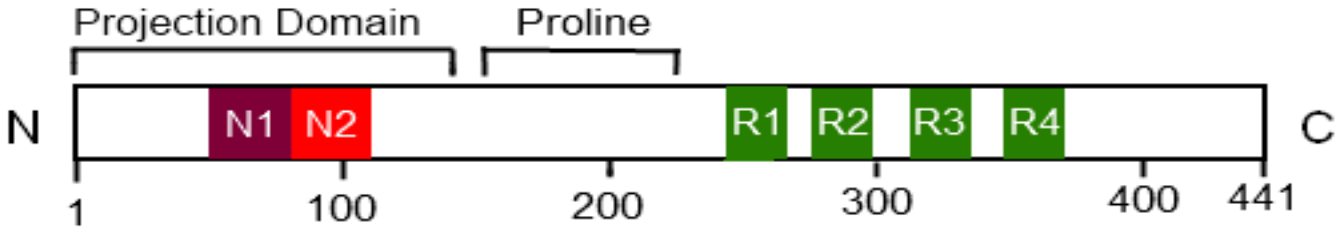
Hyperphosphorylation of tau proteins can cause the helical and straight filaments to tangle (referred to as neurofibrillary tangles). These tangles contribute to the pathology of Alzheimer's disease.





Schematic representation of six predominant isoforms and domains of tau found in human brain. The number of amino acids in each isoform and its abbreviation are indicated at the right. E2 and E3 encode respective 29-amino acid insert and each microtubule-binding repeat, designated to R1 to R4, is 18-amino acid long.
Six isoforms of tau are present in adult human brain whereas only the shortest isoform (3R/0N) of tau is present in fetal brain. The N-terminal projection domain includes the acidic and proline-rich domains, and the C-terminal microtubule-assembly domain includes microtubule-binding repeats, pseudorepeat, and C-terminal tail part.

Primary sequence analysis demonstrates that tau consists of a half-N-terminal acidic portion followed by a proline-rich region and the C-terminal tail, which is the basic part of the protein.

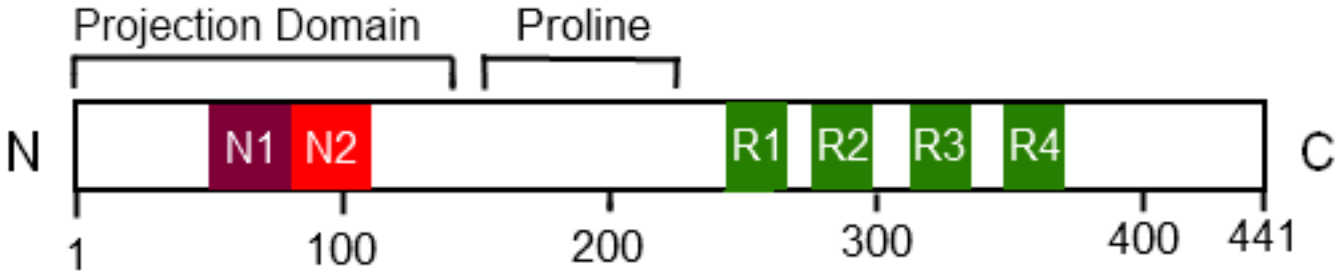


Because each of the 6 isoforms has specific physiological roles, they are differentially expressed during the development of the brain. For instance, only one tau isoform, characterized by 3R and no N-terminal inserts, is present during fetal stages, while the isoforms with one or two N-terminal inserts and 3- or 4R are expressed during adulthood.

Tau protein is present in a greater extent in axons from neurons, but it also occurs in the oligodendrocytes

The Projection Domain and Its Interaction with Other Molecules.

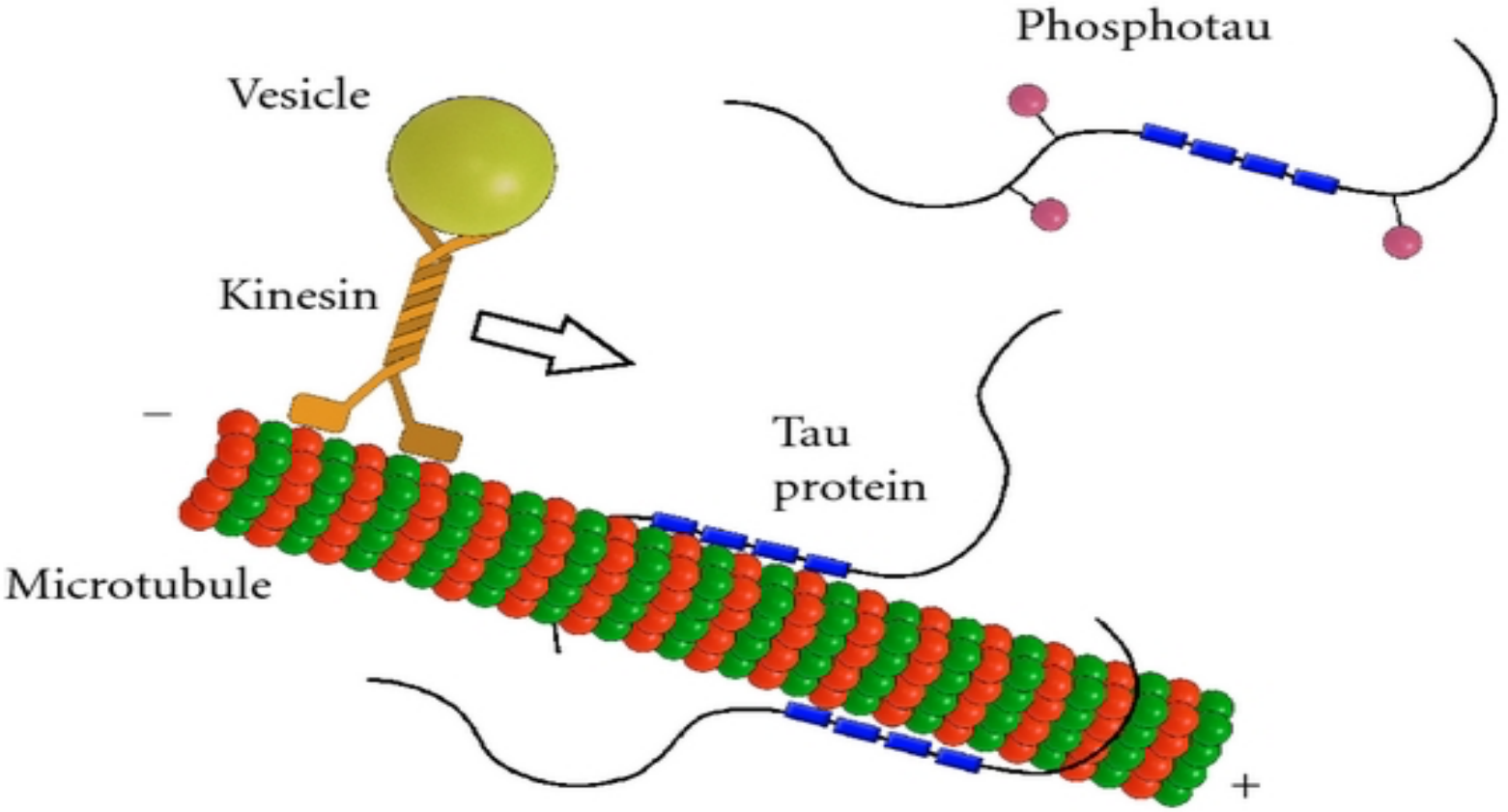
The N-terminal part is referred to as the **projection domain** since it projects from the microtubule surface where it may interact with other cytoskeletal elements and the neuronal plasma membrane

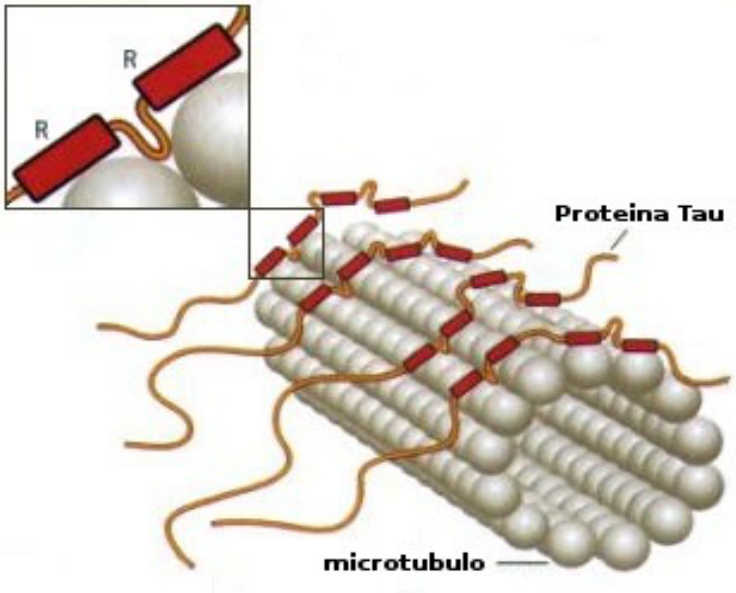


Tau binds to the microtubule through the repeats and their flanking regions

Upon binding, the N-terminal portion (< 150 residues) protrudes from the microtubule surface. For this reason, the N-terminal portion is called projection domain, which may bind to other cell components such as the plasma membrane.

As to the interactions with other cytoskeletal components, tau protein binds to spectrin and actin filaments, which may allow tau-stabilized microtubules to interconnect with neurofilaments that restrict the flexibility of the microtubule lattices.



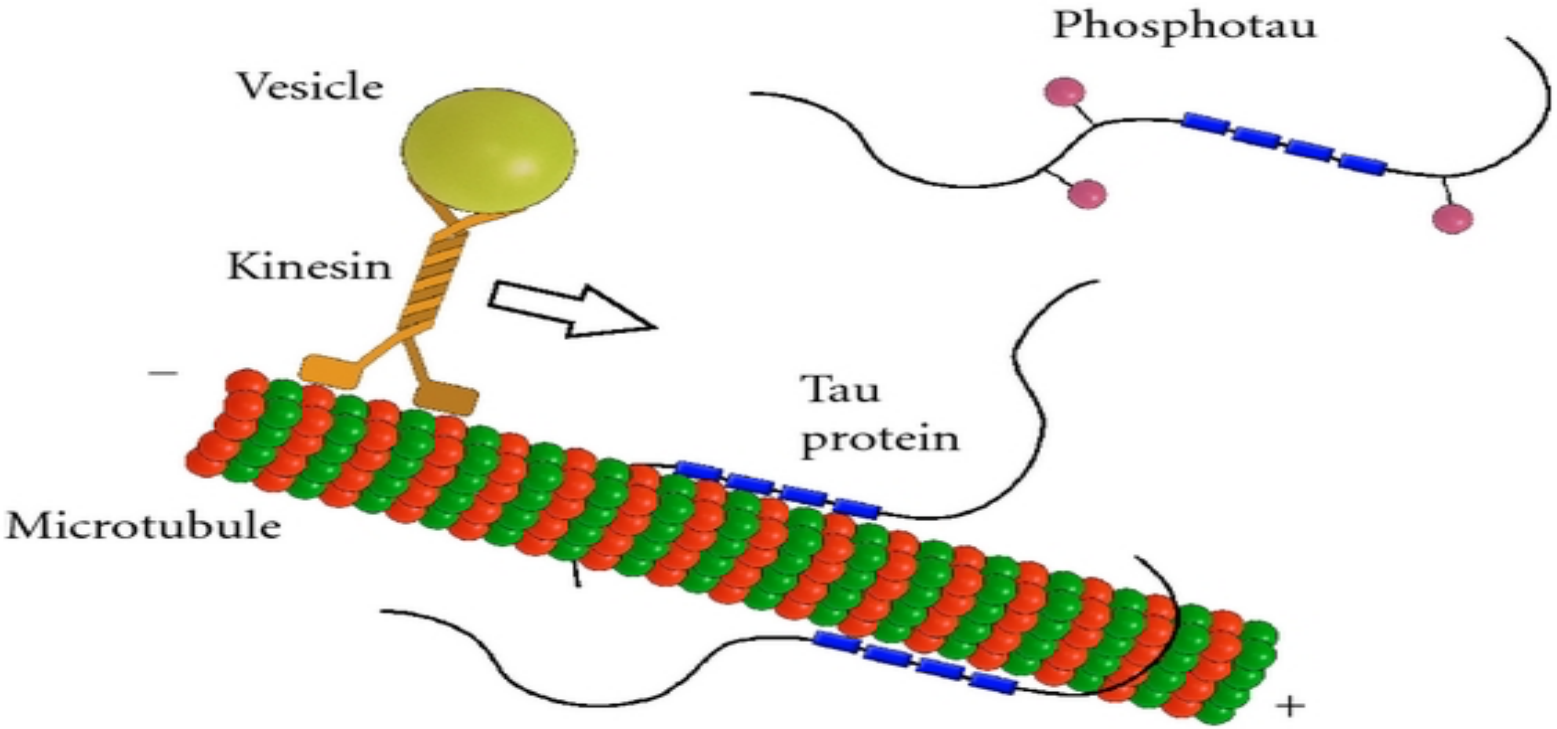


Tau and other MAPs stabilize microtubules by binding to the MT surface and promote their self-assembly from tubulin subunits

The exact binding site of Tau is not known; the uncertainty is largely due to Tau's natively unfolded structure. Recent studies have demonstrated very tight microtubule binding for the Tau fragment 208-304: this argues that the central part of Tau is aligned along protofilaments.

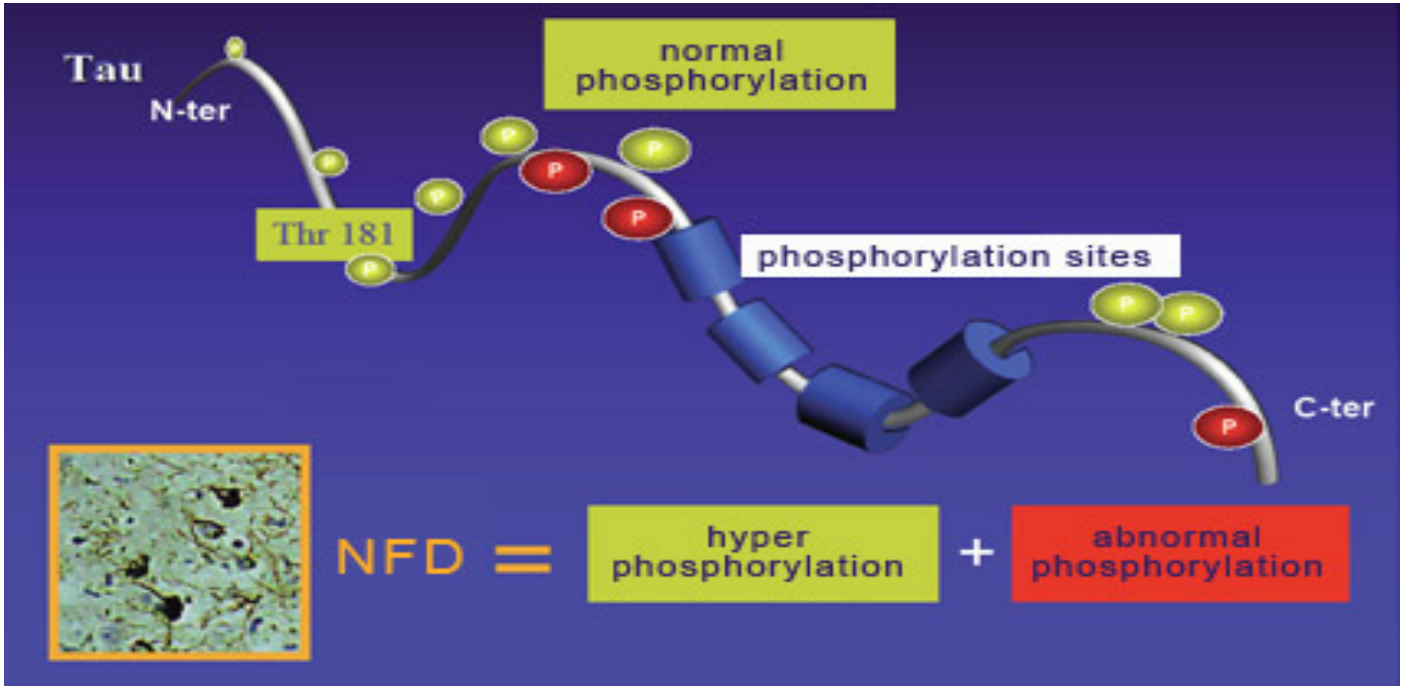
In considering functions of Tau towards microtubules, we can distinguish direct and indirect interactions. Direct interactions include the binding, stabilization and promotion of MT assembly that can be modulated by Tau and its phosphorylation. This function requires the MT binding domain (repeats + flanking domains), but not necessarily the projection domain. Another effect is the protection of microtubule ends against lenght fluctuations (dynamic instability).

Indirect interactions affect other proteins that may or may not interact with MT by themselves and may require the projection domain of Tau. An example is the spacer function of MAPs, which helps to establish a "clear zone" around microtubules in cells; another is the inhibition of Mt-dependent transport by motor proteins, which is based on the competition between motors (kinesin, dynein) and MAPs for binding sites on the MT surface.



Normal function of tau protein. Tau protein stabilizes microtubules through four tubulin binding domains (blue boxes) in case of the longest isoform. Binding of tau protein to the microtubules is maintained in equilibrium by coordinated actions of kinases and phosphatases. The phosphorylation of tau (pink balls) regulates its activity to bind to microtubules and can affect axonal transport. Tau protein may inhibit the plus-end-directed transport of vesicles along microtubules by kinesin.

In the cytosol of neurons the pools of tau protein in either phosphorylated or dephosphorylated forms are maintained in equilibrium by coordinated actions of kinases and phosphatases, respectively.
Several studies in cell lines revealed that tau protein bound to the plasma membrane is dephosphorylated



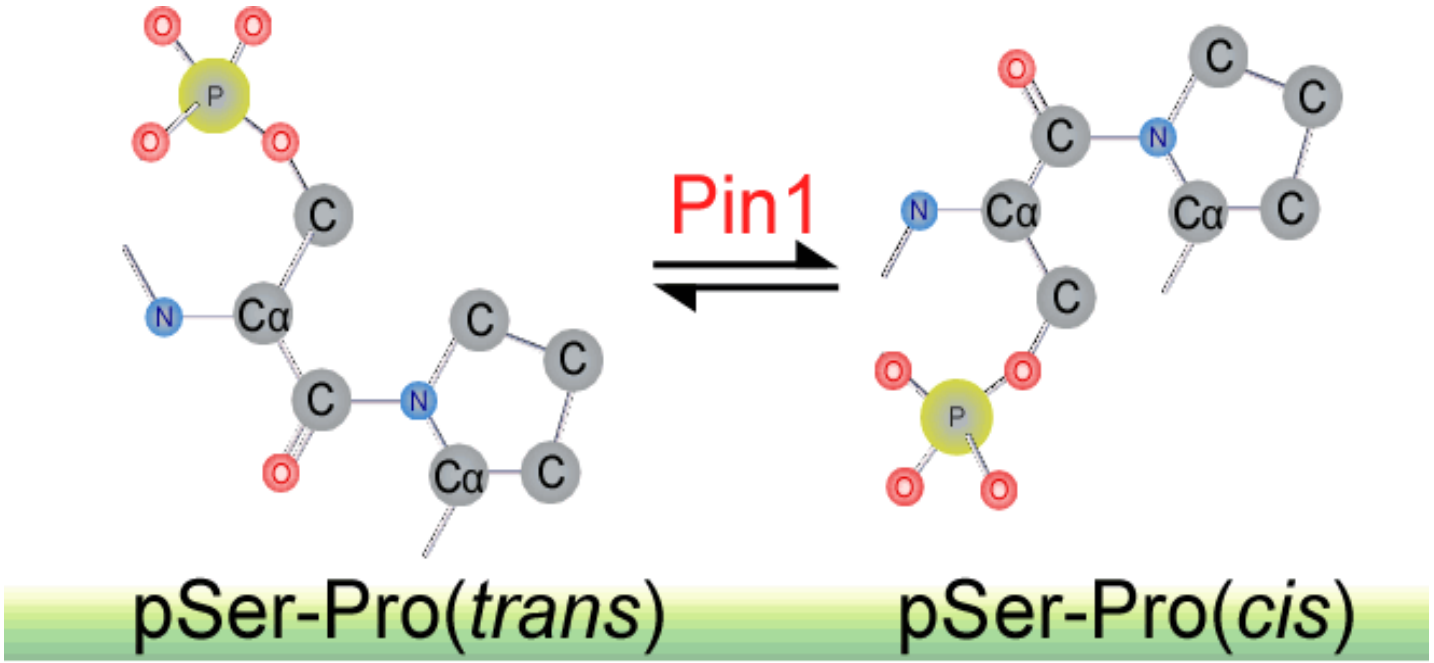
Tau binding partners.

Since Tau is very flexible and carries many charges it has the capacity to interact with many partners. By far the most important interaction of Tau is that with microtubules, but also interactions with other cytoskeletal elements have been reported as well, notably intermediate filaments and microfilaments. These interactions need not be direct, but could be mediated by other cytoskeleton-associated proteins. Binding sites of Tau have been reported for several kinases and phosphatases; the best-defined kinase interaction sites are the PXXP motifs in the proline-rich domain that can bind to SH3-containing proteins including **tyrosine kinases** Fyn, Src or Lck.

A number of studies have dealt with the interactions of Tau with the chaperone system. Proteins such as hsp70 and their binding partners are thought to control the level of soluble Tau and thus help to prevent aggregation in a phosphorylation-dependent manner. Among the non-protein interaction partners of Tau, the interactions with the plasma membrane and with nuclear components have been intriguing over years. The emerging picture is that a small fraction of cellular Tau binds to the membrane through membrane-associated proteins, for example annexins or proteins of lipid rafts.

Another molecule that interacts with tau protein is a peptidyl-prolyl cis/trans isomerase Pin 1.
It isomerizes only phosphoserine/threonine-proline motifs and binds to the tau protein after its phosphorylation on Thr231 residue.
Isomerization induces conformational changes that make tau accessible for Protein Phosphatase (PP) 2A, which in turn leads to tau dephosphorylation.
Protein Pin 1 regulates functions of tau protein and APP and is important for protection against the degeneration that occurs during the ageing process.

Activity of Pin 1 is decreased by oxidation in AD



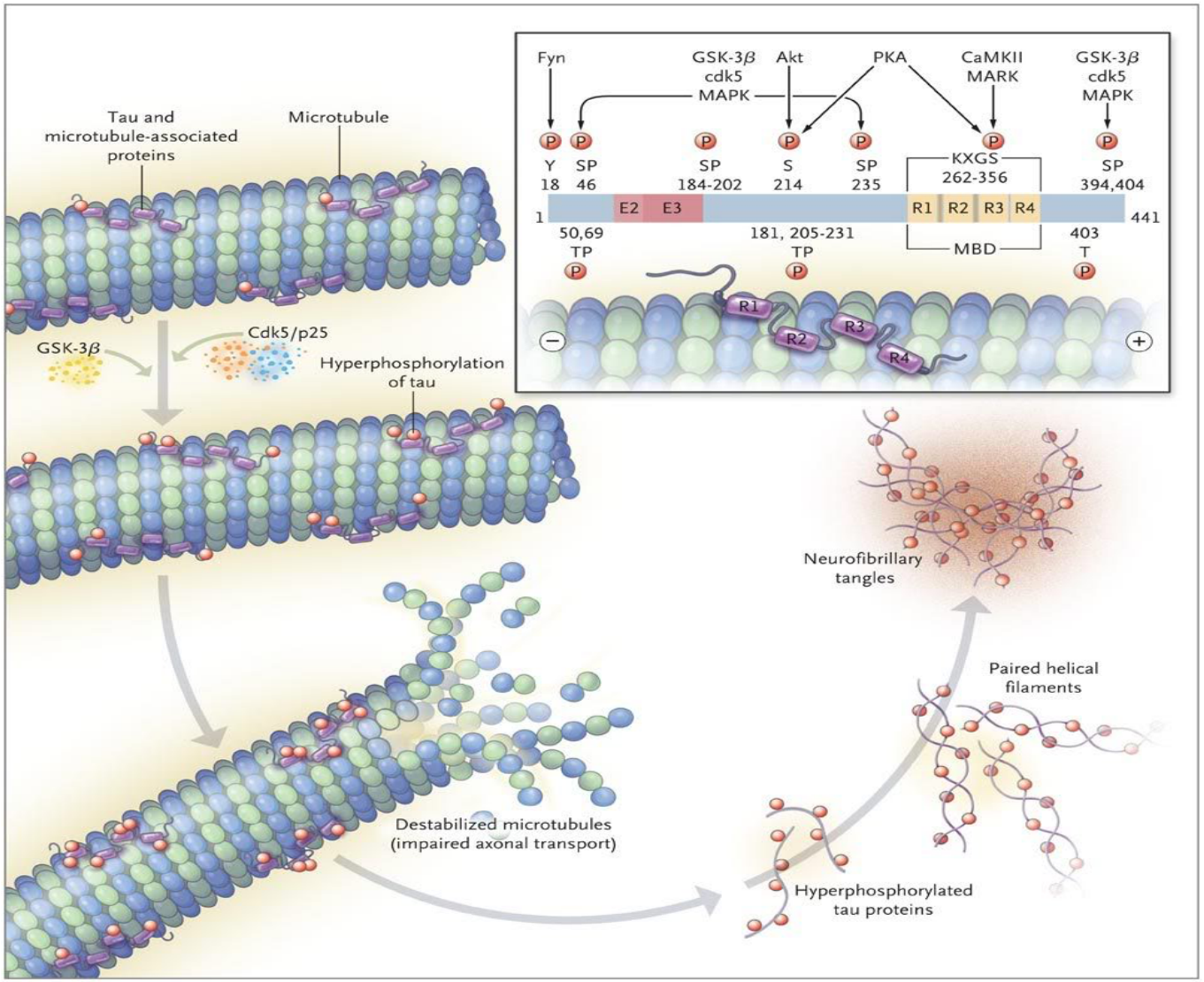
Tau Pathology

In AD, the normal role of tau protein is ineffective to keep the cytoskeleton well organized in the axonal process because the protein loses its capacity to bind to microtubules.

This abnormal behavior is promoted by conformational changes and misfoldings in the normal structure of tau that leads to its aberrant aggregation into fibrillary structures.

The mechanisms by which tau protein becomes a nonfunctional entity are in debate. Abnormal posttranslational modifications are proposed to be the main cause of this failure. In this regard, abnormal phosphorylation (hyperphosphorylation), acetylation, glycation, ubiquitination, nitration, proteolytic cleavage (truncation), conformational changes, and some other modifications have been proposed to cause the loss of normal function and the gain of pathological features of tau protein.

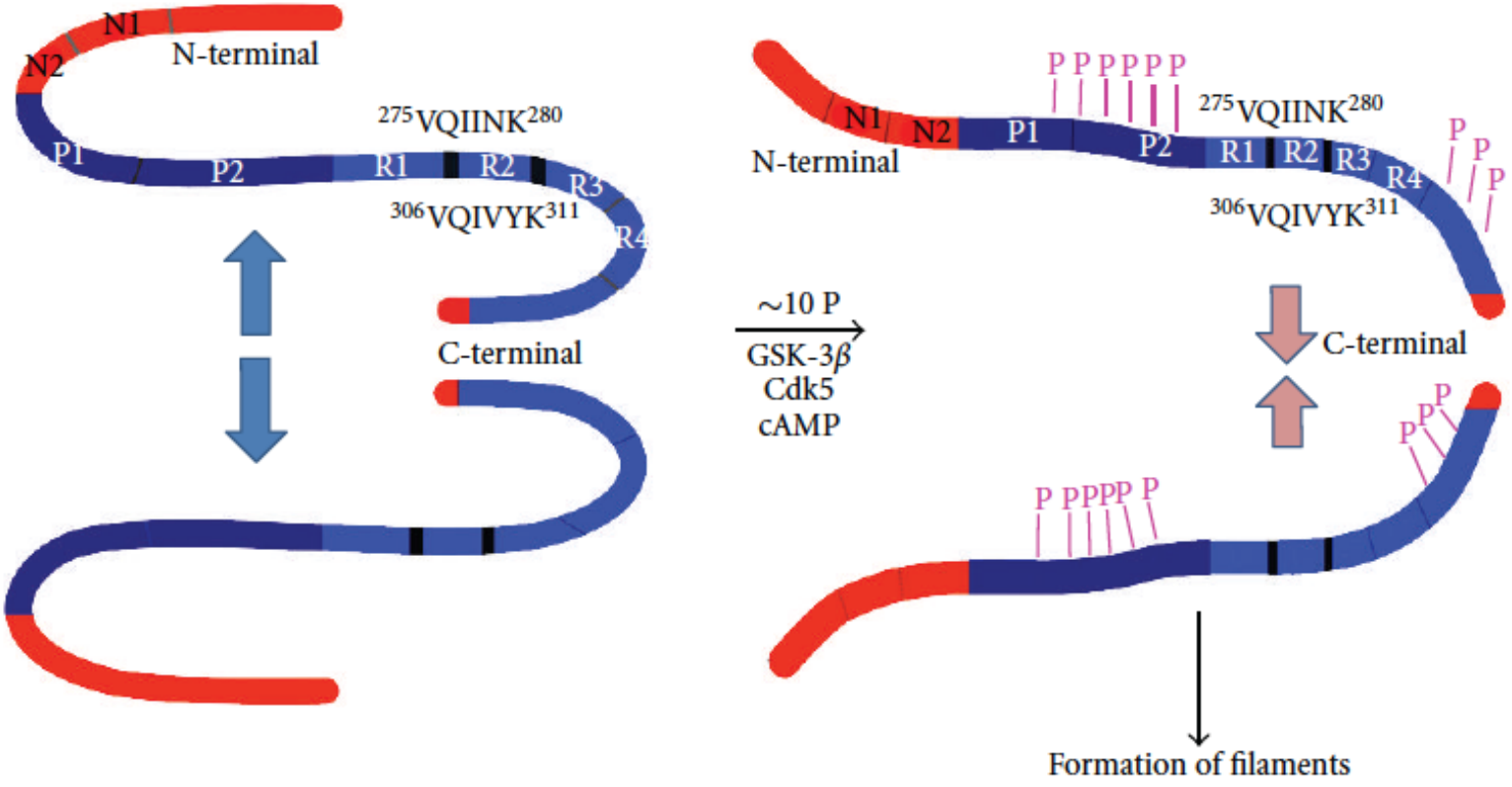
The Hyperphosphorylation of Tau Protein.



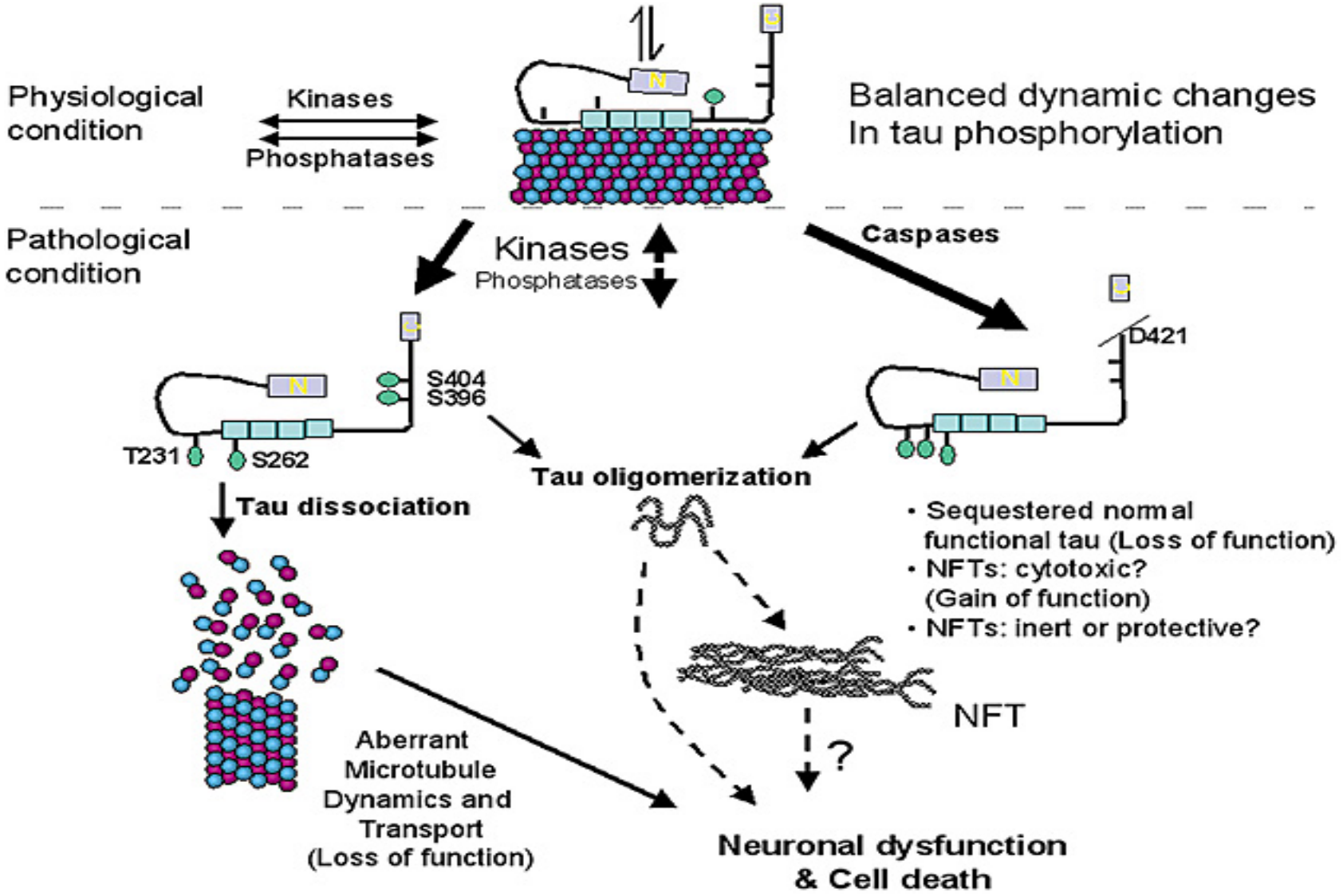
Neurofibrillary degeneration of abnormally hyperphosphorylated Tau not only occurs in AD brain but is also seen in a family of related neurodegenerative diseases, **called tauopathies**, such as frontotemporal dementia with Parkinsonism linked to chromosome 17 caused by Tau mutations, Pick's disease, corticobasal degeneration, dementia pugilistica etc

In every one of this tauopathies the neurofibrillary changes are made up of abnormally hyperphosphorylated tau and their occurrence in the neocortex is associated with dementia.

In AD brain all of the six Tau isoforms are hyperphosphorylated and aggregated.

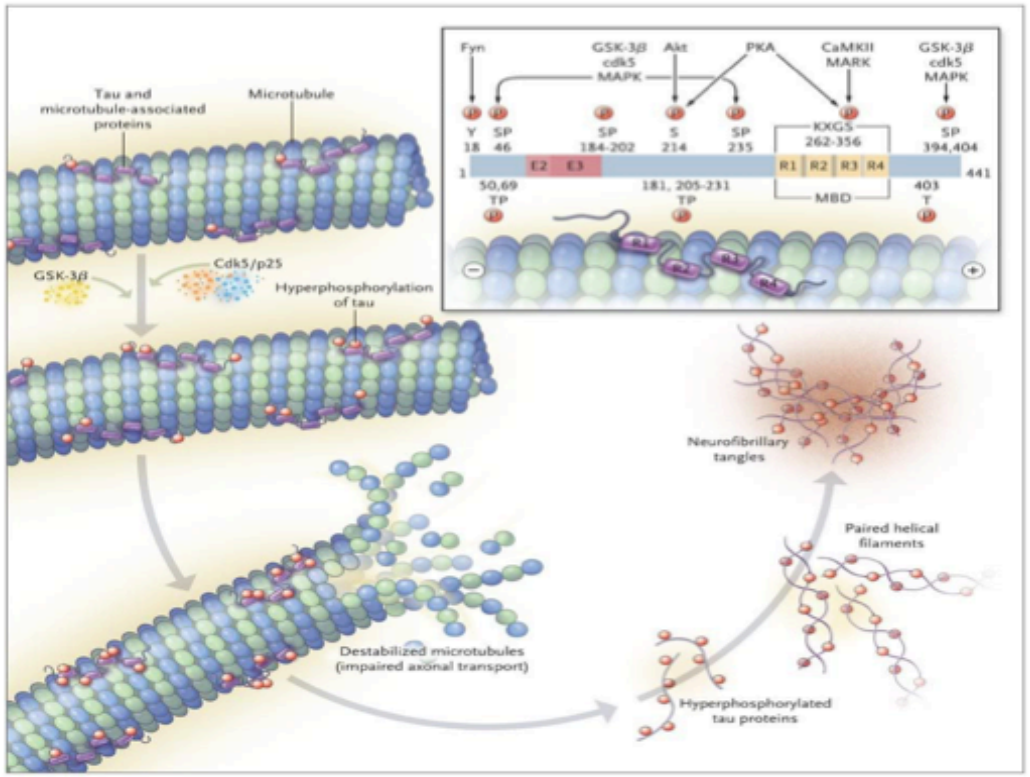


Phosphorylation of tau protein. Tau self-assembles mainly through themicrotubule binding domains/repeat R3 in 3R tau proteins and through R3 and R2 in 4R tau proteins (R2 (275VQIINK280) and R3 (306VQIVYK311) have β-structure). N-terminal and C-terminal regions to the repeats are inhibitory. Hyperphosphorylation of tau neutralizes these basic inhibitory domains, enabling tau-tau interaction (phosphorylation sites indicated by violet Ps)


Physiological condition
Kinases
Phosphatases
Balanced dynamic changes In tau phosphorylation
Pathological condition
Kinases
Phosphatases
Caspases
N
C
S404
S396
T231
S262
D421
Tau oligomerization
Tau dissociation
• Sequestered normal functional tau (Loss of function)
• NFTs: cytotoxic? (Gain of function)
• NFTs: inert or protective?
NFT
?
Aberrant Microtubule Dynamics and Transport (Loss of function)
Neuronal dysfunction & Cell death

While in the normal brain almost all tau is soluble, from AD brain this protein is recovered in three major states, i.e. soluble, oligomeric and fibrillized. There is at least as much normal cytosolic Tau in AD brain as in normal aged brain but the level of total Tau in the former is four to eight fold higher and this increase is solely in the form of the abnormally hyperphosphorylated protein. As much as 40 % of the abnormally hyperphosphorylated Tau in AD brain is present in the cytosol and not polymerized into paired helical filaments/neurofibrillary tangles.

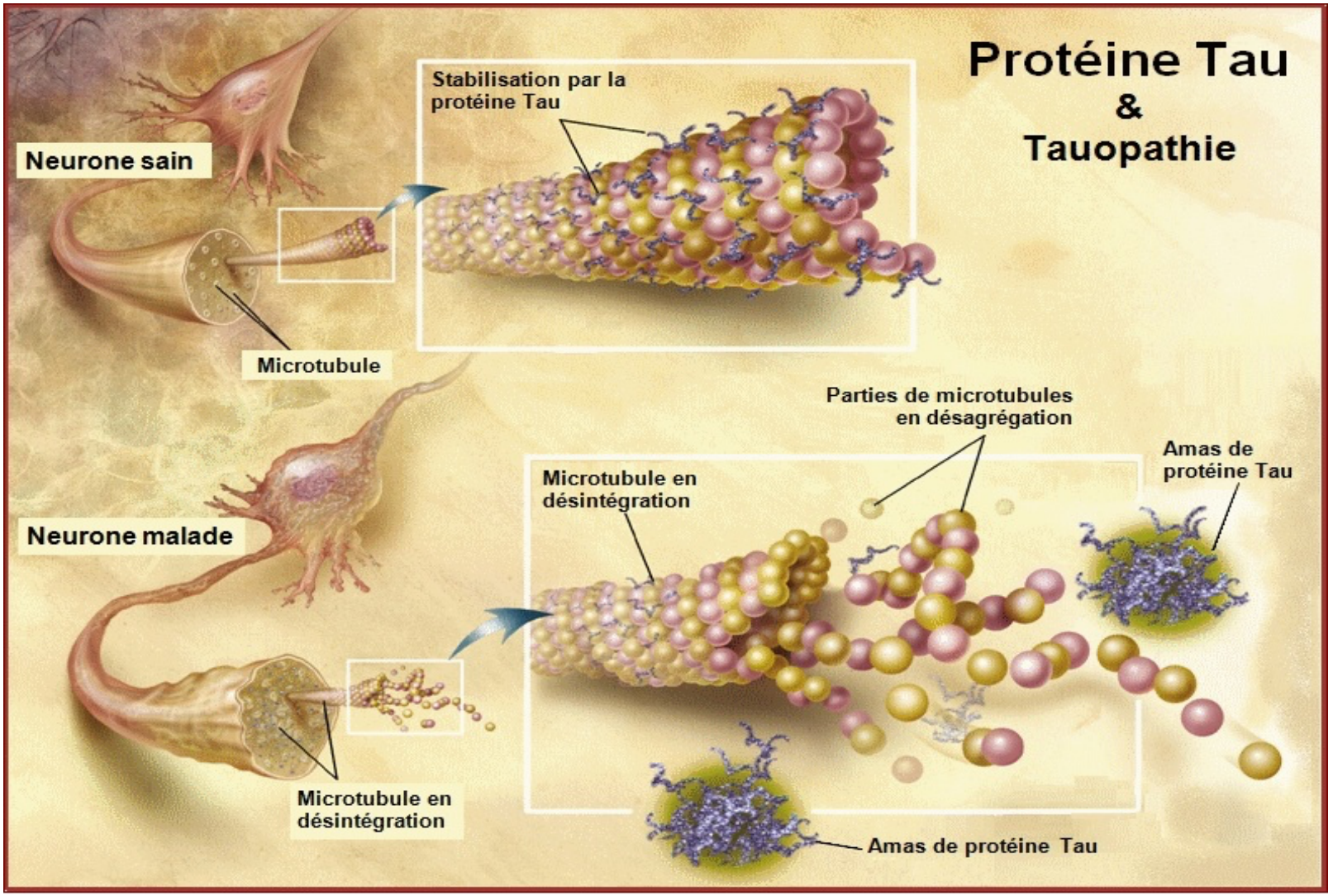
The AD cytosolic abnormally hyperphosphorylated Tau(AD P-Tau) does not bind to tubulin and promote microtubule assembly, but instead it inhibits assembly and disrupts microtubules.



This toxic property of the pathological Tau involves the sequestration of normal Tau by the diseased protein. The AD P-Tau also sequesters the other two major neuronal microtubule associated proteins MAP1A/B and MAP2. This toxic behavior of the AD P-Tau appears to be solely due to its abnormal phosphorylation because dephosphorylation of diseased Tau converts it into a normal-like protein.

In wild-type human Tau and mutated human Tau transgenic *Drosophila*, the accumulation of the AD P-Tau in the absence of its fibrillization into neurofibrillary tangles leads to neurodegeneration: this suggests that the accumulation of the cytosolic AD P-Tau, and not its aggregation, is apparently involved in behavioral impairment.

Furthermore, in vitro dephosphorylation of neurofibrillary tangles disaggregates filaments and, as a result, the Tau released behaves like normal protein in promoting microtubule assembly.
Thus, two characteristics of AD P-Tau are that it sequesters normal MAPs and disrupts microtubules and that it self-assembles into PHF (paired helical filament).


Protéine Tau
&
Tauopathie
Neurone sain
Stabilisation par la protéine Tau
Microtubule
Neurone malade
Parties de microtubules en désagrégation
Amas de protéine Tau
Microtubule en désintégration
Microtubule en désintégration
Amas de protéine Tau

In addition to abnormal hyperphosphorylation, **conformational changes and cleavage of Tau** have also been implicated in the pathogenesis of AD. The hyperphosphorylation of Tau has been found to precede both conformational changes and cleavage of this protein. Tau contains many potential cleavage sites accessible to **multiple proteases**, yelding breakdown products that could be toxic in various ways. For example cleavage of the tails by **caspases** perturbs the paperclip folding of Tau and makes it more vulnerable to aggregation.