

## news and views

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# Alzheimer's disease: Pinning down phosphorylated tau

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A defining characteristic of several neurodegenerative diseases, including Alzheimer's disease and Pick's, is the formation of filamentous deposits of a microtubule-associated protein called tau in an abnormally hyperphosphorylated form. The discovery of mutations in the *tau* gene in a condition known as 'familial frontotemporal dementia and parkinsonism linked to chromosome 17' has renewed interest in the mechanisms which dysfunction of tau causes neurodegeneration.

On [page 784](#) of this issue, Lu *et al.*<sup>1</sup> describe an intriguing interaction between phosphorylated tau and a prolyl isomerase, Pin1. Prolyl isomerases enhance the rate of *cis* to *trans* isomerization of the peptide bond on the terminal side of proline. Pin1 is an essential nuclear protein belonging to the parvulin family of prolyl isomerases. This group is distinct from two other prolyl isomerase families, the cyclophilins and the FK506-binding proteins, which are targets of the immunosuppressive drugs cyclosporine and FK506, respectively. Pin1 consists of a carboxy-terminal catalytic domain, as well as an amino-terminal protein-protein interaction region called a WW domain that specifically recognizes phosphorylated serine or threonine residues preceding a proline residue (the S/T-P motif)<sup>2,3</sup>.

In its short history, Pin1 has generated much interest because it regulates entry and progression through mitosis. It does this by interacting with a large set of mitosis-specific phosphoproteins, most of which can be detected by a monoclonal antibody called MPM2 ([ref. 3](#)). Lu *et al.*<sup>1</sup> started off armed with the knowledge that MPM2 also recognizes hyperphosphorylated tau in the brains of people with Alzheimer's disease<sup>4,5</sup>. Moreover, during mitosis tau is phosphorylated at a number of the S/T-P sites that are hyperphosphorylated in Alzheimer's disease<sup>6,7</sup>. This led the authors to examine whether phosphorylated tau interacts with Pin1. And they found that tau (phosphorylated either by a mitotic cell extract or by Cdc2 kinase) does, indeed, bind to the WW domain of Pin1.

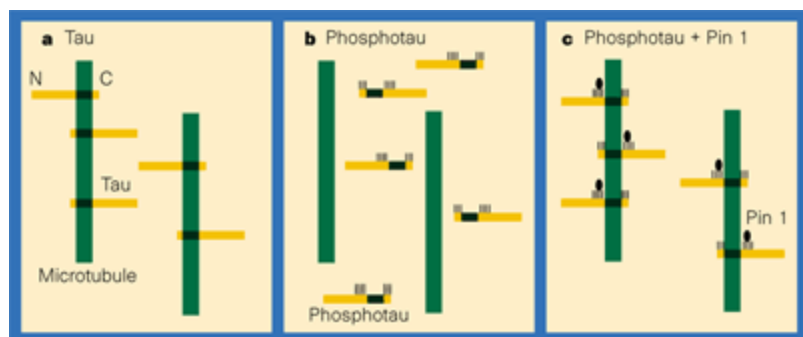
The longest isoform of tau in the human brain has 17 S/T-P sites. Of these, Lu and colleagues found that only — phosphorylated threonine 231 (T231) — was required for the interaction with Pin1. This residue is located upstream of the microtubule-binding repeats in a proline-rich region that is required for full activity of tau. The T231 residue is hyperphosphorylated in Alzheimer's disease and is also phosphorylated, to a certain extent, in normal brain<sup>8,9</sup>. This residue can also be phosphorylated by glycogen synthase kinase-3, but only after phosphorylation of serine 235 by cyclin-dependent kinase-5 or mitogen-activated protein kinase<sup>10,11</sup>.

Lu and colleagues went on to gather more evidence for the interaction between tau and Pin1. First, using a pull-down assay, they showed that Pin1 binds to hyperphosphorylated tau from the brains of people with Alzheimer's disease, but not to tau from age-matched healthy brains. Tau from normal brain is notorious for the speed with which it is dephosphorylated after death<sup>12</sup>, so it may be premature to conclude that Pin1 interacts only with hyperphosphorylated (pathological) tau.

Second, by immunoblotting, the authors detected endogenous Pin1 in the paired helical filaments (PHFs) from diseased brains. (The PHFs, which are composed of hyperphosphorylated tau, make up the pathological neurofibrillary tangles of Alzheimer's disease.) Third, using immunohistochemistry, Lu *et al.* found that recombinant Pin1 binds to pathological tau. Finally, the authors looked at localization of Pin1. In control brains they observed nuclear staining for endogenous Pin1, consistent with its known localization in non-neuronal cells. But in the brains of people with Alzheimer's disease, Pin1 staining was also associated with pathological tau in neuronal cells (although it is not clear what percentage of the tau-positive structures was also immunoreactive Pin1).

The tau protein in PHFs from the brains of patients with Alzheimer's disease is phosphorylated at more than 20 residues, many (but not all) of which are S/T-P sites<sup>13</sup>. In healthy brains, tau is heterogeneously phosphorylated between eight and ten of these residues<sup>9</sup>. Because of its abnormal hyperphosphorylation, tau from PHFs cannot bind to microtubules or promote microtubule assembly. Hyperphosphorylation of tau is believed to be an early event that precedes assembly into PHFs. Yet there is no experimental evidence linking hyperphosphorylation of tau to PHF assembly<sup>14</sup> — synthetic, paired helical-like filaments can, in fact, be produced in a phosphorylation-independent way.

To examine the functional effects of the interaction between Pin1 (Fig. 1, overleaf) and tau phosphorylated at Lu *et al.* used recombinant tau phosphorylated by Cdc2 kinase, with or without added Pin1. As expected, phosphorylated tau could neither bind microtubules nor promote microtubule assembly properly. But in the presence of Pin1, biological activity of the phosphorylated tau was restored, an effect that seemed to depend on Pin1's prolyl isomerase activity. This surprising result indicates that isomerization of the peptide bond on the amino-terminal side of proline residue 232 in tau is enough to confer biological activity upon Cdc2-tau. Moreover, it suggests that the same may be true of the hyperphosphorylated tau in Alzheimer's disease. However, Cdc2 kinase phosphorylates only some of the residues that are hyperphosphorylated in the disease, remains to be seen whether Pin1 could restore the biological activity of tau *in vivo*.



**Figure 1** Pin1 restores the ability of phosphorylated tau to bind to microtubules.

[Full legend](#)

[High resolution image and legend](#) (20k)

Lu *et al.* next compared the levels of soluble Pin1 in the brains of Alzheimer's patients with those in age-control brains. By immunoblotting and immunoprecipitation, they saw greatly reduced levels of soluble Pin1 in diseased brain. The most striking abnormality of tau in Alzheimer's disease is its assembly into filaments, and results indicate that tau filaments may sequester soluble Pin1. Depletion of Pin1 in nonneuronal cells leads to mitotic arrest<sup>2</sup>, so the observed depletion may have been related to ectopic expression of some cell-cycle markers in the brains of people with Alzheimer's disease<sup>15-17</sup>. The significance of this observation for the process is unclear, although it has been suggested that aberrant expression of cell-cycle markers may cause cell death by apoptosis.

The identification of mutations in the *tau* gene should allow us to study these questions more rigorously. Using

such mutants, it has already been shown that a reduced ability of tau to interact with microtubules may precede hyperphosphorylation and assembly into filaments<sup>14</sup>. So, compounds that reduce the pool of functionally tau could be of therapeutic value. The discovery of the interaction between Pin1 and phosphorylated tau may be the way to developing such compounds.

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