

Protein twists and turns in Alzheimer disease

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An enzyme that modifies protein structure seems to help keep Alzheimer disease at bay. The enzyme affects two proteins thought to be key to disease pathology: amyloid precursor protein (APP) and the microtubule-binding protein tau.

Research on Alzheimer disease has been driven by a focus on two proteins, tau and APP, thought to underlie the affliction. Brains of affected individuals accumulate aberrant forms of both of these proteins: tau becomes hyperphosphorylated and accumulates in neuritic aggregates and APP is cleaved to produce amyloid- β (A β) protein aggregates¹.

Many mechanisms have been proposed to explain how these proteins become modified during Alzheimer disease. Most of these mechanisms concentrate on either tau or APP, but rarely both. In a recent issue of *Nature*, Pastorino *et al.*² implicate a single protein, the prolyl isomerase Pin1, in both tau- and amyloid-related pathologies. By an apparently simple mechanism, Pin1 controls how proteins twist themselves into various isomers. Pin1 helps keep tau and APP in proper form, and its activity seems to go awry during Alzheimer disease.

Alzheimer disease, the most common and costly neurodegenerative disorder, affects nearly 2% of the population in industrialized countries. With aging populations, the incidence of this disease will continue to rise unless we can identify key molecular events in the demise of neurons that result in memory loss and reduced cognitive capacity, the hallmarks of Alzheimer disease.

Much has already been learned about how tau and APP operate during disease. The hyperphosphorylated tau fibrils prevent normal microtubule dynamics and disrupt neuronal transport mechanisms. Many kinases have been implicated in the phosphorylation of tau, including those concerned with cell-cycle control.

Metabolic shifts in APP processing underlie the generation of toxic forms of A β and the progression of amyloid plaque formation. These different forms are produced through modulation of the enzymes involved in cleavage of APP, a cell-surface protein. These enzymes, α -, β - and γ -secretases, cleave APP

at different amino acids—with resultant peptide fragments possessing benign functions (mediated by α -secretase) to toxic, amyloidogenic functions (mediated by β - and γ -secretase).

Pastorino *et al.* show that the control of APP isomerization by Pin1 affects the processing of APP into either neurotrophic or toxic cleavage products, and thus affects the eventual pathology of Alzheimer disease. The work also builds on previous studies demonstrating that Pin1 may also have a role in the control of the normal function and pathological activity of tau³.

Pin1 is a prototypic member of the peptidyl-prolyl isomerases (PPIases). These enzymes catalyze the *cis-trans* isomerization of peptide bonds N-terminal to specific phospho-Ser/Thr-Pro motifs in certain types of polypeptide chains (those found in a series of mitotically related phosphoproteins that are substrates for proline-directed kinases^{4,5}).

Pin1 was originally characterized by its interaction with a mitotic kinase (NIMA) in *Aspergillus nidulans*, in which it was shown to control cell-cycle transition from G2 to M⁶. Pin1 contains two functional domains. One domain (the N-terminal WW domain) preferentially binds to the target proteins phosphorylated during mitosis. The other domain (the PPIase domain) mediates the *cis-trans* isomerization of the peptide bond connecting the phosphorylated serine or threonine residue to the subsequent proline on the target protein.

The basal level of Ser/Thr-Pro bond isomerization in this motif is markedly attenuated upon serine-threonine phosphorylation of the protein in this motif. This phosphorylation prevents all other prolyl isomerase activity at this site, except for that of Pin1, which makes this enzyme a unique phosphorylation-specific prolyl isomerase. Hence, Pin1 may be a crucial nexus between cellular phosphorylation and the control of global protein morphology.

Interestingly, many of the targets of Pin1 activity are involved in multiple mechanisms regulating cell-cycle control, cell proliferation and apoptosis. The conformational alterations induced by Pin1 in its substrates can affect catalytic activity, phosphorylation status, protein-protein interactions, subcellular localization and eventually protein stability.

Emerging evidence indicates that a post-mitotic reactivation of mitosis may occur in Alzheimer disease, for instance, by reexpression of cyclinB-cdc2 kinase⁷. This reactivation of mitosis may result, in part, from a pathological reduction in the level of Pin1 in neurons sensitive to damage in Alzheimer disease^{8,9}.

Pin1 has been shown to possess a palliative function in Alzheimer disease, as its ability to isomerize phosphorylated tau apparently restores the ability of phosphorylated tau to bind microtubules, inhibit the mitotic inducer cdc25C and eventually promote dephosphorylation of tau by PP2A phosphatase¹⁰.

Pastorino *et al.* invoke the importance of Pin1 activity in the abnormal production of pathological forms of A β in Alzheimer disease through its effect upon APP proteolytic cleavage. Using nuclear magnetic resonance spectroscopy, the authors take an extremely close look at Pin1. They visualize, for the first time, the functional isomerizing ability of Pin1 upon a phosphorylated form of APP protein found in the brain of individuals with Alzheimer disease.

They also found that Pin1 efficiently interacts with APP in cells. The magnitude of the Pin1-APP interaction depended upon cell-cycle status and the activity of the cdc2 kinase, which phosphorylates APP upon the threonine in the Thr668-Pro motif (Fig. 1).

This phosphorylation of Thr668-Pro in APP is increased in Alzheimer disease brains and seems to lead to an alteration of APP processing, resulting in increased production of A β ¹¹. Pastorino *et al.* now provide a mechanism by which modulation of the *cis-trans* isomerization of this phosphorylated Thr668-Pro motif enhances the deleterious and attenuates the benign processing of APP.

Pastorino *et al.* show that in the basal state the phospho-Thr668-Pro motif of APP exists in a ratio of *cis* to *trans* forms that heavily favors the *trans*. The authors find that Pin1 activity dramatically enhanced the proportion of APP in the *trans* form. Supporting a role for Pin1 in APP processing, the authors provide evidence that Pin1 colocalizes and interacts with APP in vesicular structures

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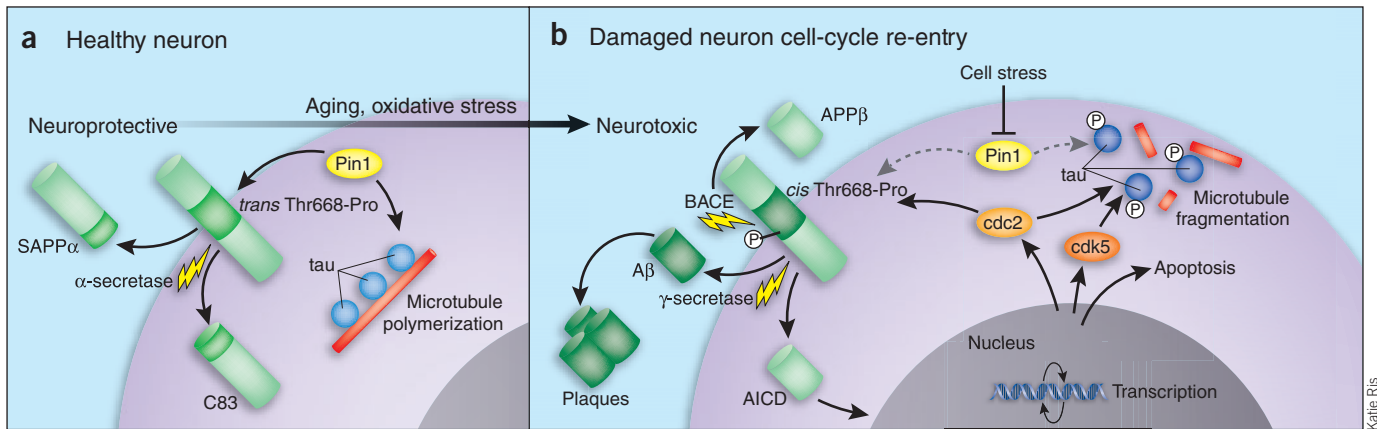


Figure 1 Pin-mediated prolyl isomerization in proteolytic processing of the amyloid precursor protein and cell cycle regulation. **(a)** Pin1 activity maintains Thr668-Pro of the amyloid precursor protein (APP) in the *trans* form, a conformation that promotes cleavage of APP by α -secretase. Cleavage of APP by α -secretase releases the neuroprotective secreted α -APP (SAPP α) and the C83 intracellular domain, and prevents production of neurotoxic amyloid- β peptide (A β). In addition, Pin1-mediated prolyl isomerization of tau facilitates polymerization of microtubules, thereby permitting normal cytoskeletal dynamics and processes such as axonal transport. **(b)** Levels of active Pin1 are decreased by unclear mechanisms (perhaps cell stress), resulting in increased amounts of the *cis* Thr668-Pro isomer of APP. This conformation of APP facilitates cleavage by β - and γ -secretases, resulting in the generation of neurotoxic A β and the formation of amyloid plaques. Lack of prolyl isomerization of tau compromises its ability to promote microtubule polymerization, resulting in cytoskeletal disruption and self-aggregation of tau into neurofibrillary tangles. Both APP and tau are phosphorylated by kinases involved in the cell-cycle re-entry, for example, *cdc2* or *cdk5*. Finally, by acting on proteins involved in cell-cycle regulation, Pin1 may maintain neurons in a postmitotic state. Loss of Pin1 activity in Alzheimer disease may, together with DNA damage, trigger cell-cycle reentry and apoptosis. The APP intracellular domain (AICD) generated by γ -secretase cleavage may also induce proapoptotic effects through alterations in gene expression.

(Fig. 1). Cellular overexpression of Pin1 induced a reduction in amyloidogenic processing of APP, resulting in increased production of secreted α -APP and decreased levels of A β . Corroborating a role of Pin1 in controlling processing of APP, in mitotically active breast cancer cells from Pin1-deficient mice, there was an increased production of the toxic A β 42 and a reduced production of the neurotrophic secreted α -APP compared with cells from wild-type mice.

Therefore, in response to cell-cycle alterations in vulnerable neurons, the actions of Pin1 would act to maintain phospho-Thr688-Pro APP in the *trans* form, thus promoting benign processing of APP into secreted α -APP. But under the conditions of increased oxidative stress, believed to occur in Alzheimer disease, Pin1 may be downregulated. Downregulation would favor the *cis* form of phospho-Thr668-Pro APP and, as the authors suggest, there would be a concomitant increase in production of toxic A β 42.

The role of Pin1 in the metabolism of APP was reinforced using combined mutant mice lines. In aged Pin1-deficient mice (15 months), compared to wild-type mice, the authors demonstrated a selective elevation of insoluble A β 42 (A β 40 was unchanged). This elevation was not seen in younger (6-month-old) Pin1-deficient mice. Pin1-deficient mice were also bred with a strain possessing a mutation in APP that promotes APP's amy-

loidogenic processing to produce APP-Tg2576 mice, which were also deficient in Pin1. In this strain, a statistically significant increase in toxic A β 42 was seen as early as 6 months old, much earlier than in the Pin1-deficient mice (Fig. 1). Therefore, a loss of Pin1 exacerbated the generation of toxic A β 42 in the double-mutant mice, reinforcing the palliative role of Pin1 in the generation of Alzheimer disease pathology.

The authors therefore propose that Pin1 can not only prevent the formation of pathological conformations of tau, but that it can also prevent pathological proteolytic processing of APP by inducing a subtle conformation change in the protein. Thus, Pastorino *et al.* may have mechanistically unified, through Pin1, the two major pathologies of Alzheimer disease.

If the authors' view is true, then Pin1 and the prolyl isomerization process are new targets for therapeutic intervention in Alzheimer disease. Other findings, however, suggest a darker side of Pin1, involving interactions with proteins other than APP and tau. The involvement of Pin1 in cell-cycle regulation suggests a potential role in neuronal death because cell-cycle re-entry is considered a trigger for apoptosis in postmitotic neurons¹². In addition, some actions of Pin1 can trigger neuronal apoptosis; for example, its interaction with BH3-only proteins, which induce mitochondrial

membrane-permeability transition¹³. The possibility that the reduced levels of Pin1 in Alzheimer disease brain are the result of the selective death of neurons that express high levels of Pin1 therefore merits further investigation.

Finally, the findings of Pastorino *et al.*² raise questions about the role of Pin1 and prolyl isomerization in regulating secretase activities. Pin1 alters either the interaction of the components of the secretase complexes with APP or the interaction of protein intermediates with APP and the secretase complexes. A detailed investigation into the 'interactome' of *cis* or *trans* phospho-Thr668-Pro APP would likely yield a more complete answer as to how the shift of APP processing occurs.

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