The prolyl isomerase PIN1: a pivotal new twist in phosphorylation signalling and disease

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Abstract | Protein phosphorylation regulates many cellular processes by causing changes in protein conformation. The prolyl isomerase PIN1 has been identified as a regulator of phosphorylation signalling that catalyses the conversion of specific phosphorylated motifs between the two completely distinct conformations in a subset of proteins. PIN1 regulates diverse cellular processes, including growth-signal responses, cell-cycle progression, cellular stress responses, neuronal function and immune responses. In line with the diverse physiological roles of PIN1, it has also been linked to several diseases that include cancer, Alzheimer's disease and asthma, and thus it might represent a novel therapeutic target.

Pro-directed kinase
A protein kinase that phosphorylates certain Ser/Thr residues that precede a Pro residue (Ser/Thr-Pro motifs).

Peptidyl-prolyl *cis/trans* isomerase
A member of a large superfamily of enzymes that catalyse *cis*/*trans* isomerization of Pro imidic peptide bonds by reducing the energy barrier between these two states.

Polo-like kinase
A family of protein kinases that regulate spindle assembly, mitotic entry and chromosome segregation.

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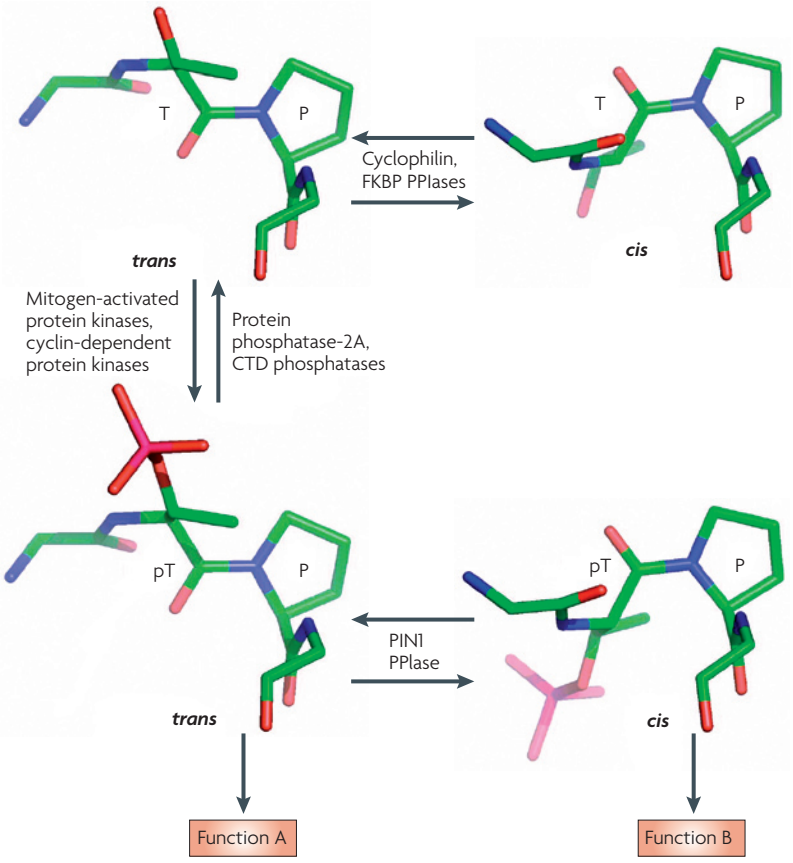
Protein phosphorylation is a key cellular signalling mechanism that induces changes in protein conformation[1,2]. For example, the phosphorylation of many Tyr residues and some Ser/Thr residues acts as a signal to recruit proteins into signalling networks or to place enzymes close to their substrates. Similarly, the phosphorylation of enzymes such as glycogen phosphorylase and certain protein kinases induces conformational changes in their catalytic domains, which allows substrates to access the catalytic sites that are otherwise inaccessible. However, it remains largely unknown how many other phosphorylation events actually regulate cell signalling and whether they can be further regulated after phosphorylation.

Ser or Thr residues that precede Pro (Ser/Thr-Pro) are a major regulatory phosphorylation motif in cells[2–6]. Enzymes that are responsible for such phosphorylation belong to a large superfamily of Pro-directed protein kinases, which include cyclin-dependent protein kinases (CDKs), extracellular signal-regulated kinases (ERKs), stress-activated protein kinases/c-Jun-N-terminal kinases (SAPKs/JNKs), p38 kinases, glycogen synthase kinase-3 (GSK3) and Polo-like kinases (PLKs). These kinases have a crucial role in diverse cellular processes such as cell growth regulation, stress responses and neuronal survival, as well as in human diseases such as cancer and Alzheimer's disease (AD)[2–6]. The unique stereochemistry of Pro means that it can adopt two completely different conformational states (BOX 1). However, the significance of this with respect to the fact that Pro is often found next to phosphorylated Ser/Thr residues was not appreciated for a long time[6,7].

A breakthrough in understanding the conformational importance of Pro-directed phosphorylation motifs was the discovery of a unique and conserved peptidyl-prolyl *cis/trans* isomerase (PPIase), PIN1 (protein interacting with NIMA (never in mitosis A)-1)[6,8–10]. PPIases are evolutionarily conserved enzymes that catalyse the *cis*/*trans* isomerization of peptidyl-prolyl peptide bonds[11] (BOX 1). Although PIN1 belongs to the parvulin subfamily of PPIases, it is the only PPIase that specifically recognizes phosphorylated Pro-directed Ser/Thr (pSer/Thr-Pro) peptide sequences[6,8–10]. The identification of PIN1 as a phosphorylation-specific PPIase led to the hypothesis of a new signalling mechanism, whereby PIN1 catalytically regulates the conformation of substrates after their phosphorylation to further control protein function[9,10,12,13]. Subsequent studies have shown that PIN1-catalysed conformational regulation can have a profound impact on many key proteins that play a part in the regulation of cell growth, genotoxic and other stress responses, the immune response, germ cell development, neuronal differentiation and survival[6,7,14–23]. PIN1 has emerged as a novel molecular timer that modulates its multiple targets at various steps of a given cellular process to synergistically control the amplitude and duration of a cellular response or process. Importantly, PIN1 is tightly regulated by multiple levels and its deregulation has an important role in a growing number of pathological conditions, including cancer, AD, ageing, asthma and microbial infection[6,7,14–23]. Recent studies have also shown that phosphorylation-independent prolyl isomerization functions as a molecular timer to regulate a growing

Box 1 | **PIN1-catalysed conformational switch of pSer/Thr-Pro motifs**



Proline is unique among amino acids in its ability to adopt either the *cis* or *trans* state of the backbone torsion angle, due to its five-membered ring in the peptide backbone (see figure). The local environment of Pro can influence the relative free energies of the *cis* and *trans* isomeric states, leading to wide variations in the ratio of *cis*/*trans* populations in different proteins. Although most structures require Pro to adopt one or the other isomer in the context of native protein folds, several recent structures show the presence of both populations for specific Pro residues[30]. Due to the relatively large energy barrier (ε^{u} = 14–24 kcal mol^{-1}), uncatalysed isomerization is a rather slow process but can be greatly accelerated by peptidyl-prolyl *cis*/*trans* isomerases (PPIases). These enzymes have a role in protein folding and include the well-studied cyclophilins (Cyps) and FK506-binding proteins (FKBPs), which are cellular targets for the immunosuppressive drugs cyclosporin A and FK506, respectively; FKBPs are also a target for the immunosuppressant and anticancer agent rapamycin. However, it turns out that the action of these drugs does not involve the inhibition of PPIase activity, but rather the inhibition of calcineurin phosphatase activity or TOR (target of rapamycin) kinase activity[139]. Moreover, all eight Cyps and four FKBPs are dispensable individually and together for viability in budding yeast[35]. Therefore, PPIases were thought to perform non-essential cellular roles, and doubt was cast on their value as therapeutic targets. The discovery of the phosphorylation-specific prolyl isomerase PIN1 has cast a new light on the cellular importance of prolyl *cis*/*trans* isomerization. Recent studies have uncovered that both phosphorylation-dependent and -independent prolyl isomerization can function as a molecular timer in several biological and pathological processes[30].

CTD phosphatase, RNA polymerase-II C-terminal domain phosphatase.

number of biological and pathological processes, including cell signalling, ion-channel gating, gene expression and phage infection[11,24–30].

Here, we review the molecular and structural basis for PIN1-catalysed post-phosphorylation regulation, discuss the significance of such a regulatory mechanism in human physiology and pathology, and explore the potential of this mechanism for new disease diagnosis and therapeutic interventions.

Unique switch of Pro-directed phosphorylation

Pro is unique in its ability to adopt either the *cis* or the *trans* conformation; while its uncatalysed isomerization is rather slow, it can be catalysed by PPIases[11] (BOX 1). Although PPIases can control the interconversion kinetics of *cis*/*trans* isomerization — an intrinsic conformational switch — they were thought to perform non-essential cellular roles, and the significance of this enzymatic activity as an important regulatory mechanism in human physiology and pathology was not known until the discovery of PIN1 (REFS 7,9,29,30).

PIN1 specifically isomerizes pSer/Thr-Pro motifs, which is especially important because Pro-directed kinases and phosphatases are conformation-specific and act only on the *trans* conformation[31–33]. Furthermore, phosphorylation dramatically slows down the already slow rate of isomerization of Ser/Thr-Pro bonds and renders the phosphopeptide bond resistant to the catalytic action of conventional PPIases[9]. Pro-directed phosphorylation also induces local structural changes that make it accessible to further modifications[34].

PIN1 and its homologues are the only enzymes known so far that efficiently isomerize such Pro-directed phosphorylation motifs[6,7,9]. This is supported by the fact that in budding yeast, the PIN1 homologue Ess1 is the only essential gene out of a total of 13 PPIase genes[8,35]. PIN1 is also essential in some organisms such as *Aspergillus nidulans*[36] and *Candida albicans*[37], but not in others such as fission yeast[38] and mice[39], although *PIN1*-knockout animals do have many interesting phenotypes[6]. A possible explanation is that PIN1 may have some overlapping functions with other PPIases[40,41]. However, PIN1 only targets to a small subset of Pro-directed phosphorylation sites and other phosphorylation-specific PPIases may exist[6]. Indeed, there are at least three PIN1 homologues in plants[42], two in *Drosophila melanogaster*, and phosphorylation-specific PPIases can be detected in PIN1-deficient cells (K.P.L., unpublished observations). Furthermore, mRNA encoding a gene that is highly related to *PIN1*, *PIN1L*, has been identified in mice, although its corresponding protein and function have not yet been identified[43].

Dynamics of the PIN1-catalysed switch

PIN1 has a two-domain structure that consists of an N-terminal WW domain (named after two invariant Trp residues) and the C-terminal PPIase domain (FIG. 1a). Early structure–function analyses *in vitro* and *in vivo* have revealed that the unique substrate specificity of PIN1 towards specific pSer/Thr-Pro motifs results from interactions provided by both of these domains — a 'double-check' mechanism[5,6,8–10,12,33]. The WW domain binds only to specific pSer/Thr-Pro-motifs, which are often crucial regulatory phosphorylation sites in PIN1 substrates[12,33,44–48] (FIG. 1b). WW domain binding targets PIN1 to substrates in different subcellular compartments, whereas the PPIase domain isomerizes specific

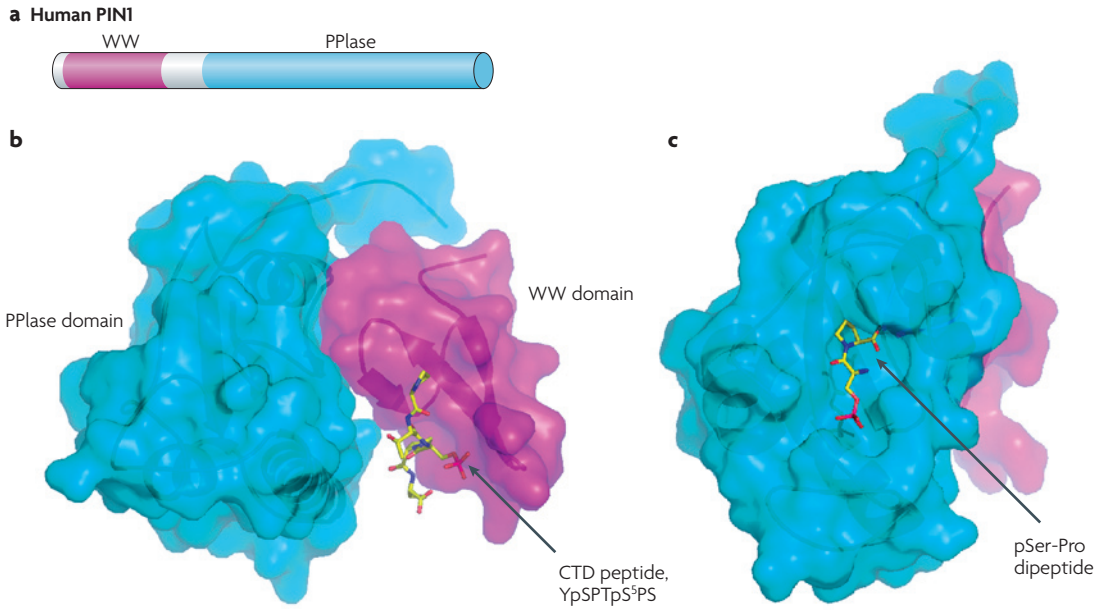


Figure 1 | **Structural basis for the unique specificity of PIN1 towards specific pSer/Thr-Pro motifs. a** | The domain architecture of human PIN1 (protein interacting with NIMA (never in mitosis A)-1). PIN1 contains an N-terminal WW domain, which mediates binding to specific pSer/Thr-Pro motifs, and a C-terminal peptidyl-prolyl *cis/trans* isomerase (PPIase) domain that catalyses isomerization of specific pSer/Thr-Pro motifs in the substrate. **b** | X-ray structures of PIN1 in a complex with a C-terminal domain (CTD) peptide (YpSPTpS[5]PS) in the WW domain. Ser16, Arg17 and Tyr23 in the WW domain are responsible for binding the phosphate of pSer[5] (the fifth residue in the peptide) and the aromatic rings of Tyr23 and Trp34 form an aromatic clamp, which accommodates the ring atoms of Pro[6], the sixth residue[49]. **c** | X-ray structure of PIN1 in complex with a pSer-Pro dipeptide modelled in the PPIase domain[10]. A set of conserved catalytic residues in all PIN1-type PPIases project outward from the barrel structure and define the binding pocket for the proline and the peptide bond that undergoes *cis/trans* isomerization. The side chains of Lys63, Arg68 and Arg69 form a basic cluster that sequesters pSer in the substrate. Lys63 is conserved in PIN1- and parvulin-type PPIases and is involved in basic catalysis[9,33]. In contrast, Arg68 and Arg69 are conserved only in PIN1-type PPIases and are responsible for the unique phosphorylation specificity of PIN1-type PPIases[9,33].

pSer/Thr-Pro motifs to regulate protein function by controlling their conformations[12,33] (FIG. 1c).

Various subsequent structural analyses have validated this basic principle, and have also revealed how PIN1 dynamically binds to its substrates and regulates their conformations[49–56]. Nuclear magnetic resonance (NMR) analysis of the action of PIN1 on amyloid precursor protein (APP) has revealed the dynamic regulation of the intracellular domain of APP between the two completely distinct structures[53]. APP possesses a Thr668-Pro motif that exists in *trans* before phosphorylation. The *cis* conformation appears only after phosphorylation due to local structural constraints, with ~10% of the phosphorylated motifs existing in the *cis* conformation[57] (FIG. 2). PIN1 binds to the pThr668-Pro motif in the *cis* and *trans* conformations and also accelerates the isomerization rate >1000-fold (in the millisecond timescale) compared with the typical uncatalysed reaction (which is in the minute timescale), with the catalysed *cis* to *trans* rate being 10-fold faster than the reverse rate[53]. Therefore, PIN1 would rapidly re-establish *cis/trans* equilibrium if either population was suddenly depleted in the cell.

The structures of PIN1 in complex with its binding phosphopeptides have confirmed the phosphorylation-dependent interaction[49,50]. It remains unclear why PIN1 binds only to specific pSer/Thr-Pro motifs in certain proteins but it may be due to combined primary (and other) structural determinants. The sequence that is crucial for the PIN1-binding specificity is located in the WW domain at an intrinsically flexible loop, the flexibility of which changes upon ligand binding, suggesting that sequence-specific dynamics are important for PIN1 substrate specificity[55]. It is also unknown how WW and PPIase domains are coordinated to act on PIN1 substrates. NMR studies have shown that WW and PPIase domains are loosely connected modules, but their mobility can be differently modulated by different phosphopeptides[51]. Furthermore, the WW domain can increase or inhibit PIN1 isomerase activity depending on whether a peptide substrate is phosphorylated on a single site or on multiple sites *in vitro*[52], although the biological significance of these findings is unclear. The findings that many substrates contain a single phosphorylation target for PIN1 (see Supplementary information S1 (table)) suggest that when it is targeted to the substrate by the WW domain, the PPIase domain would have to act on the same pSer/Thr-Pro motif to accelerate its isomerization. Alternatively, when a PIN1 substrate is multiphosphorylated or in a multiprotein complex, the WW domain and PPIase domain might act on different pSer/Thr-Pro motifs in the same protein or in different ones. Further studies are needed to address these important questions.

WW domain
A protein-interacting module that contains ~38 amino acid residues folded into a three-stranded β-sheet structure. The domain name is derived from two conserved Trp residues that are spaced 20–22 residues apart within the consensus sequence.

Amyloid precursor protein
A transmembrane glycoprotein that is sequentially cleaved to generate Aβ peptides in Alzheimer's disease.

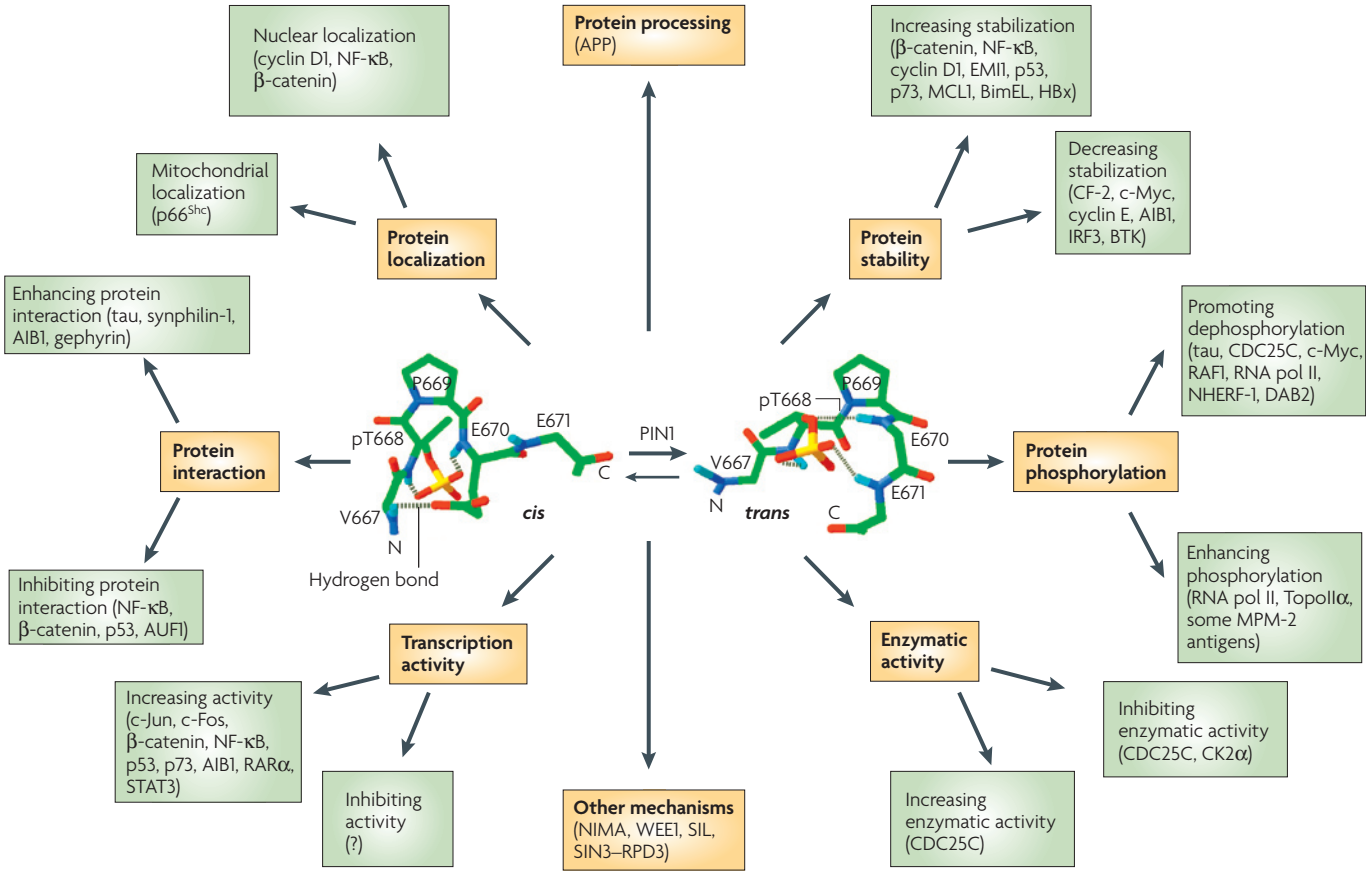


Figure 2 | **PIN1-catalysed prolyl isomerization regulates a spectrum of target activities.** Phosphorylation dramatically reduces *cis*/*trans* isomerization of certain regulatory Ser/Thr-Pro motifs between the two completely distinct *cis* and *trans* conformations, but converts the peptide bonds as substrates specifically for PIN1 (protein interacting with NIMA (never in mitosis A)-1). PIN1 greatly accelerates *cis* to *trans* or *trans* to *cis* isomerization depending on specific target sites and local structural constraints, thereby regulating protein conformation after phosphorylation between two distinct structures, as shown for amyloid precursor protein (APP) visualized by nuclear magnetic resonance spectroscopy. Such conformational changes have profound effects on phosphorylation signalling by regulating a spectrum of target activities. For example, PIN1-catalysed *cis* to *trans* isomerization of APP promotes non-amyloidogenic APP processing because the *cis* conformation might prefer amyloidogenic APP processing. Similarly, PIN1-catalysed *cis* to *trans* prolyl isomerization promotes protein dephosphorylation or enhances protein phosphorylation, probably because phosphatases such as PP2A can only dephosphorylate pSer/Thr-Pro motifs in *trans*. PIN1 can increase or reduce protein turnover, which might depend on whether the pSer/Thr-Pro motif is in *trans* or *cis*; for example, F-box proteins, which target ubiquitin-mediated proteolysis in a phosphorylation-dependent manner, bind to pSer/Thr-Pro motifs only in *trans*[138]. Therefore, the effects of PIN1 probably depend on the conformation of individual Ser/Thr-Pro motifs after phosphorylation, which might explain why PIN1 often has opposite effects depending on substrates and, potentially, on phosphorylation sites even in the same substrates. AIB1, amplified in breast cancer-1; AUF1, AU-rich element RNA-binding protein-1; BimEL, apoptosis facilitator (also known as B-cell lymphoma protein-2 (BCL2)-like-11); BTK, Bruton's tyrosine kinase; CF-2, a transcription factor; CK2α, casein kinase-2α; c-Myc, transcription factor; DAB2, disabled homologue-2; EMI1, early mitotic inhibitor-1; HBx, hepatitis B virus X-protein; IRF3, interferon-regulatory factor-3; MCL1, myeloid cell leukaemia sequence-1; MPM-2, monoclonal antibody that recognizes mitosis-specific and cell-cycle-regulated phosphoproteins; NF-κB, nuclear factor κB; NHERF-1, Na^+/H^+ exchanger regulatory factor; p53 and p73, tumour suppressors; $p66^{Shc}$, 66 kDa isoform of the growth factor adaptor Shc; RAF1, v-raf-1 murine leukaemia viral oncogene homologue-1; RARα, retinoic acid receptor-α; RNA pol II, RNA polymerase-II; SIL, stem cell leukaemia (SCL) interrupting locus; SIN3–RPD3, histone deacetylase; STAT3, signal transducer and activator of transcription-3; TopoIIα, topoisomerase-IIα; WEE1, mitosis inhibitor protein kinase.

Regulation of PIN1 function

In contrast to most other constitutively active PPIases[11], PIN1 function is tightly regulated at multiple levels under physiological conditions, as expected given the tight regulation of Pro-directed phosphorylation signalling. First, the subcellular localization and function of PIN1 are dependent on the availability of its substrates, which must be phosphorylated on specific Ser/Thr-Pro motifs[58]. With a few exceptions (such as neurons, in which PIN1 expression is induced upon neuronal differentiation[44,59,60]), PIN1 expression is generally correlated with cell proliferative potential in normal human tissues, but is further upregulated in many human cancers[45–47,61,62]. Indeed, PIN1 expression

is subject to E2F-mediated transcriptional regulation in response to growth factors[6,63]. Moreover, PIN1 is one of the genes that is most drastically suppressed by the tumour suppressor BRCA1 (breast cancer-1, early onset)[64].

PIN1 is also regulated by multiple post-translational modifications. PIN1 phosphorylation is regulated in a cell-cycle-dependent manner, including phosphorylation on Ser16 in the WW domain, which abolishes the capability of PIN1 to interact with its substrates[58]. In addition, PIN1 has been shown to be phosphorylated on Ser65 by Polo-like kinase-1 (PLK1), which increases the stability of PIN1 by reducing its ubiquitylation[65]. Finally, PIN1 enzymatic activity is inactivated by oxidative modifications that occur in the early stages of AD[17,66,67]. It remains a challenge to identify these and other PIN1 post-translational modification sites and their modifying enzymes and significances.

Recent studies have uncovered that a growing number of processes are governed by PIN1-catalysed post-phosphorylation conformational changes, rather than initial phosphorylation *per se* (FIG. 2). This highlights PIN1 as an enzyme of crucial physiological and pathological importance. Strikingly, PIN1 often uses multiple mechanisms to modulate individual substrates, and/or regulates multiple targets at various steps of a given cellular process to synergistically drive the cell in one direction under certain conditions. The following sections explore the role of PIN1 in cell growth regulation, in cellular stress responses, in neuronal function and in immune responses.

PIN1, the cell cycle and growth

Human PIN1 was originally identified by its ability to interact with NIMA — a mitotic kinase that is phosphorylated on multiple Ser/Thr-Pro motifs during mitosis in *Aspergillus nidulans* — and to suppress the ability of NIMA to induce mitotic catastrophe in budding yeast[8]. This functional interaction has now been confirmed in *A. nidulans*, in which PIN1 is also essential[36]. PIN1 has a crucial role in mitotic regulation in yeast, mammalian cells and *Xenopus laevis*[8,58,68–71]. Moreover, PIN1 acts on several mitosis-specific phosphoproteins, which are also recognized by MPM-2, a monoclonal antibody that recognizes conserved phosphoepitopes in mitosis-specific phosphoproteins, suggesting that PIN1 might coordinate mitotic events[9,68,69].

Role of PIN1 in coordinating mitosis. Progression through the different phases of the cell cycle is driven by the timely activation and inactivation of different Pro-directed cyclin-dependent kinases (CDKs)[3]. For example, mitotic events are triggered by activation of cyclin B–CDC2, which leads to Pro-directed phosphorylation of several hundred proteins[72]. Notably, PIN1 has been shown to be a key regulator of mitotic events by acting on numerous substrates, such as CDC25C and WEE1, which are the activating phosphatase and inhibitory kinase of cyclin B–CDC2, respectively[12,33,73] (see Supplementary information S1 (table)). PIN1 can inhibit or increase the catalytic activity of CDC25C depending on the conditions used to induce CDC25C phosphorylation, presumably on different phosphorylation sites[68,71], and it can promote CDC25C dephosphorylation[33]. In *Xenopus laevis*, Pin1 can also inhibit the function of the essential Wee box in the non-catalytic domain of Wee1, which allows inactivation of *Xenopus* somatic Wee1 at M phase[73]. In addition, PIN1 stabilizes EMI1 (early mitotic inhibitor-1), which prevents the anaphase-promoting complex (APC) from acting on cyclin A and B during S and G2 phases, allowing the coordination of S and M phases[74].

A recent study shows that PIN1 localizes to chromatin in the G2–M phase by interacting with topoisomerase (Topo)-IIα[75]. Moreover, purified PIN1 and cyclin B–CDC2 kinase are by themselves sufficient to induce chromatin condensation in *Xenopus* extracts, presumably by increasing phosphorylation of TopoIIα by cyclin B–Cdc2 and promoting its binding to DNA[75]. Therefore, PIN1-dependent regulation of many mitotic proteins such as CDC25C, EMI1, WEE1 and TopoIIα might provide a means for temporally and spatially orchestrating the activity of mitotic proteins into an abrupt wave of signalling that proceeds in a synchronous manner. Indeed, inhibition or deletion of PIN1 leads to mitotic entry, chromatin condensation and mitotic catastrophe[8,58].

PIN1 and the G1–S phase transition. A growing body of evidence indicates that PIN1 also plays a major part in the G0/G1–S phase transition. PIN1 levels in normal cells are significantly elevated at the G1–S transition[63], and PIN1 substrates that control the G0/G1–S transition have been identified (see Supplementary information S1 (table)). For example, PIN1 increases the transcription and protein stability of cyclin D1, a key regulator of G1–S phase progression[45–48]. By contrast, PIN1 destabilizes the transcription factor c-Myc and cyclin E, which are important for the G1–S transition at different time points[76,77]. Also, *Pin1*-knockout mouse embryonic fibroblasts (MEFs) display several defects in G0–G1 and G1–S transitions[39,77], which indicates the importance of PIN1 in these phases of the cell cycle.

A recent study revealed a role of PIN1 in the coordination of centrosome duplication and DNA synthesis during the cell cycle[78]. Centrosome duplication must be coupled to the events of the nuclear cell cycle and this strict coordination is regulated by multiple pathways involving Pro-directed phosphorylation[79,80]. PIN1 localizes to centrosomes and its ablation drastically delays centrosome duplication[78]. By contrast, PIN1 overexpression induces centrosome amplification, chromosome aneuploidy and tumorigenesis *in vitro* and *in vivo*[78]. Because PIN1 is elevated at the G1–S transition[63] and is also regulated by cell-cycle-specific phosphorylation[58], these results suggest that PIN1 is normally required for the coordination of centrosome duplication and DNA synthesis[78].

PIN1, growth-signalling pathways and oncogenesis. Pro-directed phosphorylation is a central signalling mechanism in regulating growth pathways and oncogenesis[1,2]. In fact, many oncogenes and tumour suppressors are

E2F
A family of transcription factors.

Mitotic catastrophe
A type of cell death that is linked to abnormal activation of the mitotic kinases due to deficient cell-cycle checkpoints.

Wee box
A small region in the N-terminal regulatory domain of *Xenopus laevis* somatic Wee1 that is required for both normal kinase activity and mitotic inactivation of the kinase.

Anaphase promoting complex
A multiprotein complex that is activated during mitosis to initiate anaphase and functions as an E3 ubiquitin ligase to ubiquitylate proteins for degradation by the 26S proteasome.

G2–M phase
A stage in the cell cycle whereby chromosomes that have been duplicated during S phase undergo compaction in preparation for mitosis.

Topoisomerase
A class of enzymes that alter the supercoiling of DNA. This allows the occurrence of DNA replication or transcription, or chromatin condensation.

Centrosome
A major microtubule-organizing structure in animal cells that determines the organization of the mitotic spindle poles that segregate duplicated chromosomes between dividing cells.

Box 2 | **Prevalent PIN1 overexpression in human cancers**

Cancer is a collection of diseases that have the common feature of uncontrolled cell proliferation. A central signalling mechanism that controls cell proliferation and malignant transformation is Pro-directed phosphorylation. The phosphorylation-specific prolyl isomerase PIN1 was originally found to be overexpressed in tissues and cell lines of breast cancer[45,46], which has been confirmed and expanded by comparing PIN1 expression in 60 different human tumour types and their corresponding normal tissues[61]. PIN1 overexpression is observed in most common cancers including prostate, breast, lung and colon cancer, but not in some others such as renal cancer[61]. In prostate cancer, the overexpression of PIN1 positively correlates with a higher probability of and a shorter period to tumour recurrence following radical prostatectomy[140]. PIN1 overexpression has also been shown to have some prognostic value in other cancers including colon cancer[141] and lung cancer[142]. Comparison of PIN1 expression with other tumour markers in human cancer tissues has led to the discovery that PIN1 is a crucial catalyst for oncogenesis[22,45,46,48,78,143].

directly regulated by Pro-directed phosphorylation and/or can trigger signalling pathways involving Pro-directed phosphorylation. Recent studies have demonstrated that *PIN1* is prevalently overexpressed in human cancers, that *PIN1* overexpression is correlated with poor clinical outcome of patients with cancer (BOX 2) and that PIN1 has a pivotal role in multiple oncogenic signal pathways[7,22,81,82] (FIG. 3).

PIN1 can increase cyclin D1 gene expression by multiple mechanisms, including activation of the c-Jun/c-Fos, β-catenin/T-cell transcription factor (TCF) and nuclear factor (NF)-κB transcription factors[45,46,48,83]. Furthermore, PIN1 can directly bind to phosphorylated cyclin D1 and stabilize its protein stability[47]. Moreover, *Pin1*-knockout mice display a phenotype that resembles cyclin-D1-knockout mice in some tissues such as the mammary gland[47]. Together, these effects can lead to deregulated cell cycling. PIN1 is a target gene for the E2F family of transcription factors, which can become overexpressed in response to HER2 (also known as ERBB2 or Neu) or Ras activation[63]. This suggests that a positive feedback loop may exist, which is supported by the findings that *PIN1* overexpression can greatly enhance the transformed phenotype that is induced by oncogenic Neu and Ras, whereas PIN1 inhibition has opposite effects[63]. Furthermore, PIN1 also suppresses a negative feedback mechanism of Ras by promoting the dephosphorylation and, therefore, the activation of Raf, a downstream effector of Ras[84]. Moreover, *Pin1* knockout in mice prevents oncogenic c-Neu or v-Ha-Ras, but not c-Myc, from inducing breast cancer[85], as does knockout of cyclin D1 (REF. 86), which highlights the specificity of PIN1 action in oncogenic signalling pathways.

The role of PIN1 in cancer is further supported by recent findings that *PIN1* overexpression correlates with centrosome amplification in human cancer tissues and that *PIN1* overexpression induces centrosome amplification, aneuploidy and tumorigenesis *in vitro* and *in vivo*[78]. These results indicate that oncogenes increase *PIN1* expression, which in turn is required for the activation of multiple upstream oncogenic signalling pathways and for the progression of downstream cell cycle events such as centrosome amplification (FIG. 3). In addition, PIN1 is required for activation of the transcription factor STAT3 (signal transducer and activator of transcription-3), which is constitutively activated in various tumours and also functions as an oncogene[87]. Together, these results suggest that the inhibition of PIN1 might offer an attractive new option for treating cancer (BOX 3).

There are, however, some discrepancies between *in vitro* and *in vivo* studies regarding the loss of PIN1 function in oncogenesis. In breast, prostate and liver cancer cells, overexpression of PIN1 activates the β-catenin pathway leading to the increased transcription of c-Myc[46,83,88], whereas in MEFs that are derived from *Pin1*-deficient mice with an isogenic B6 genetic background, c-Myc and cyclin E1 are found to be more stable because their protein turnover is regulated by PIN1 (REFS 76,89). These *Pin1*-null MEFs appear to have increased genomic instability and to be more prone to Ras-dependent transformation after being immortalized by p53 inactivation, leading to a proposal that PIN1 is a conditional tumour suppressor in the isogenic B6 background[23,89]. However, this hypothesis has been refuted by the recent findings that in double *Pin1/p53*-knockout mice in the B6 background (in which the instances of malignant tumours would be expected to be increased if PIN1 is a conditional tumour suppressor in this genetic background), the instances of malignant tumour formation are completely prevented, although these mice show increased normal thymocyte proliferation due to defective differentiation[90]. Furthermore, the removal of PIN1-binding sites in the crucial Pro-rich domain of p53 does not affect the ability of p53 to inhibit tumour development induced by oncogenes in mice[91]. In addition, *Pin1* knockouts are highly effective in preventing oncogenic Ras or Neu from inducing breast cancer *in vitro* and *in vivo*[63,85]. These results do not support PIN1 as a tumour suppressor and highlight the importance of using both *in vitro* and *in vivo* relevant models to study PIN1-dependent regulation; they also indicate a requirement for further evaluating if PIN1 could have any tumour-suppressing function.

PIN1 and cellular stresses

Many protein kinases that are activated in response to various stresses are Pro-directed kinases, which include SAPKs/JNKs, p38 MAPKs and ERKs[92,93]. PIN1 has been shown to regulate the function of several key proteins that are involved in various cellular stress responses. For example, in response to genotoxic insults, PIN1 binds to phosphorylated p53 and p73 and increases p53 protein stability by inhibiting its binding to the ubiquitin E3 ligase MDM2 or increases p73 protein stability by promoting its binding to and acetylation by p300 (REFS 94–97). PIN1 can also enhance the DNA-binding activity and transcriptional activity of both p53 and p73 towards their target genes[94–97]. Functionally, PIN1 is required for maintaining DNA damage checkpoints, which protect cells from DNA damage-induced apoptosis[94,95], and can also induce apoptosis by enhancing pro-apoptotic gene expression[96,97].

Recently, PIN1 has been shown to regulate mitochondrial import of the 66 kDa isoform of the growth factor adaptor Shc ($p66^{Shc}$) in response to oxidative damage[98].

Ubiquitin E3 ligase
An enzyme that facilitates the attachment of ubiquitin onto substrates.

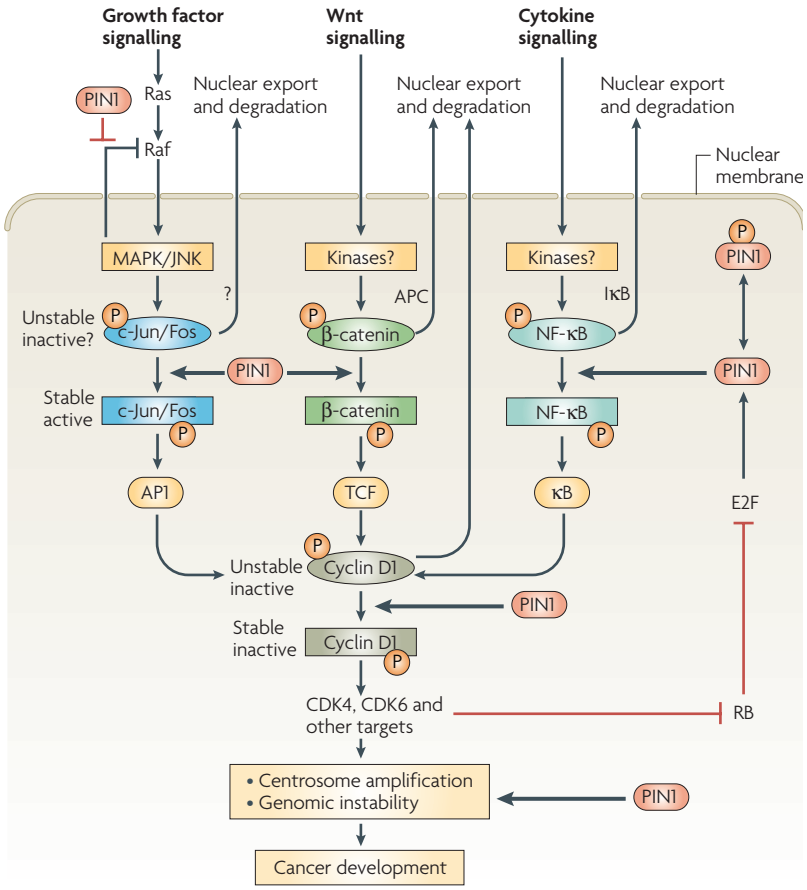


Figure 3 | **PIN1 promotes oncogenesis by regulating multiple oncogenic signalling at various levels.** Overexpression of PIN1 (protein interacting with NIMA (never in mitosis A)-1) can function as a catalyst for oncogenesis at various levels of multiple oncogenic pathways. PIN1 can increase cyclin D1 transcription by modulating at least three different signalling pathways. PIN1 targets to the pSer63/73-Pro motifs in c-Jun and multiple pSer/Thr-Pro motifs in c-Fos, which thereby increases activator protein-1 (AP1) transcriptional activity. Moreover, PIN1 promotes dephosphorylation of Raf kinase, which is usually phosphorylated and inactivated by mitogen-activated protein kinases (MAPKs) in a negative feedback mechanism. This prevents Raf kinase from being inactivated, further prolonging Raf activation. PIN1 also targets to the pThr246-Pro motif in β-catenin and to the pThr254-Pro motif in the p65/RelA subunit of nuclear factor (NF)-κB, thereby preventing these proteins from being inhibited and exported to the cytoplasm for ubiquitin-mediated degradation by their respective inhibitors (β-catenin from the adenomatous polyposis coli (APC) protein or the androgen receptor, NF-κB from IκB (inhibitor of NF-κB)). These PIN1 effects increase expression of target genes for AP1, β-catenin and NF-κB, which include cyclin D1. PIN1 also directly targets to the pThr286-Pro motifs in cyclin D1 and stabilizes cyclin D1 by preventing its nuclear export and ubiquitin-mediated degradation. The fact that PIN1 transcription is activated by the E2F–retinoblastoma (RB) pathway following oncogenic activation suggests the existence of a positive feedback loop, in which oncogenic activation leads to an increase in PIN1, which in turn enhances cyclin D1 transcription and stabilization. In addition, PIN1 overexpression disrupts the tight coupling of centrosome duplication with DNA synthesis, which leads to centrosome amplification, chromosome instability and tumorigenesis. CDK4, cyclin-dependent kinase-4; CDK6, cyclin-dependent kinase-6; JNK, c-Jun-N-terminal kinase; TCF, T-cell transcription factor.

Tau
A microtubule-binding protein that promotes microtubule assembly.

p66^{Shc} translates oxidative damage into cell death by producing reactive oxygen species within mitochondria, and is involved in regulation of lifespan[99]. Activation of protein kinase Cβ by oxidative conditions induces phosphorylation of p66^{Shc} and triggers mitochondrial accumulation of the protein after it is recognized by PIN1 (REF. 98), suggesting that PIN1 might be involved in oxidative stress and ageing[98]. However, protein kinase Cβ is not known to be a Pro-directed kinase and the PIN1 targeting site(s) in p66^{Shc} remain to be determined.

PIN1 activity is inactivated by oxidative stresses[67]; *Pin1* knockout in mice causes a range of premature ageing phenotypes[47,62] and prevents oncogenesis induced by p53 knockout[90]. These results suggest that PIN1 is involved in the response to different cellular stresses, especially during ageing, although much remains unknown about its biological or pathological significance *in vivo*.

PIN1 in neuronal function and survival

PIN1 is expressed in most neurons at unusually high levels[8,44–46]. In fact, PIN1 expression is induced during neuron differentiation[60]. Although the physiological function of PIN1 in neurons remains largely unknown, PIN1 regulates several proteins that are important for neuronal function and survival, including tau[33,44,59,60], APP[53], gephyrin[100] and myeloid cell leukaemia sequence-1 (MCL1)[101] (see Supplementary information S1 (table)). In the case of tau, phosphorylation disrupts its ability to bind to microtubules and promote their polymerization, which is crucial for maintaining the physiological function of healthy neurons[5]. However, PIN1-catalysed isomerization can restore the biological function of phosphorylated tau directly or indirectly by promoting tau dephosphorylation[33,44,59,60]. Likewise, PIN1 can also promote non-amyloidogenic APP processing, therefore producing neurotrophic αAPPs and reducing neurotoxic amyloid-β (Aβ) peptides[53]. PIN1 also enhances the ability of gephyrin to bind the β-subunit of glycine receptors, which is important for maintaining a high concentration of inhibitory glycine receptors juxtaposed to presynaptic releasing sites[100]. In addition, PIN1 protects against spinal cord injury by preventing JNK3-induced MCL1 degradation, cytochrome *c* release and apoptosis *in vitro* and *in vivo*[101], which are opposite to the *in vitro* findings that PIN1 acts on BimEL (also known as B-cell lymphoma protein-2 (BCL2)-like-11) to induce apoptosis[102]. Most significantly, PIN1 dysfunction has a prominent role in age-dependent neurodegeneration, especially in AD.

PIN1 protects against neurodegeneration in AD. The neuropathological hallmarks of AD are extracellular senile plaques comprising Aβ peptides (which are derived from APP) and intracellular neurofibrillary tangles that are composed of hyperphosphorylated tau. However, their molecular link(s) remain elusive[103–107] (BOX 4). A growing body of evidence indicates some common features between the normal cell cycle and degenerated AD neurons, especially mitotic phosphorylation on certain Ser/Thr-Pro motifs[5,108,109]. Of significance is the incorporation of mitotic MPM-2 phosphoepitopes into tangles and their appearance before tangle formation and neurodegeneration in AD brains. Phosphorylation of APP on the Thr668-Pro motif is also increased in AD brains and can elevate

Box 3 | **PIN1 as a new opportunity for disease diagnosis and therapies**

A growing body of evidence suggests that the phosphorylation-specific prolyl isomerase PIN1 is an attractive new diagnostic and therapeutic target for some diseases, especially cancer. PIN1 is prevalently overexpressed in human cancers and its expression levels correlate with poor clinical outcome[45,46,61,140]. PIN1 inhibitors may simultaneously block multiple oncogenic signalling pathways and thereby overcome cancer-cell resistance to inhibition of specific kinases or phosphatases[82]. The known PIN1 inhibitors include the natural product juglone[144], the small molecule PiB[145] and others[146], but their specificity and potency remain a major concern. Nanomolar non-natural peptidic PIN1 inhibitors have been identified[147,148], but their poor cell-membrane permeability limits their use. Therefore, there is an urgent need for the development of highly specific and potent PIN1 inhibitors, which ideally do not cross the blood–brain barrier to avoid possible side-effects on neurons. The findings that *cis* and *trans* pSer/Thr-Pro motifs in certain proteins have different biological functions and/or pathological consequences suggest that the detection of these unique conformations might offer an attractive opportunity for disease diagnosis. Indeed, antibodies that recognize Alzheimer's disease (AD)-specific tau phosphoepitopes, such as TG3, have been used to diagnose AD and track disease progression[130,149]. Given that PIN1 genetic polymorphisms correlate with reduced PIN1 levels in blood cells and with increased risk in AD[118], and that PIN1 is inactivated by oxidative modifications at early stages of AD[17], it would be interesting to examine whether PIN1 could be used as an AD biomarker. More speculatively, preventing PIN1 inactivation or raising PIN1 function in vulnerable neurons could delay or even prevent neurodegeneration in AD.

Aβ secretion *in vitro*[4,110]. Thus, increased Pro-directed phosphorylation is a common feature in both tangle and plaque pathologies, pointing to a possible role for PIN1 (REF. 5.)

In AD brains, the level of cytoplasmic PIN1 is increased and colocalizes with neurofibrillary tangles, which results in the depletion of soluble PIN1 (REFS 44,111–113), although PIN1 might be a compensatory protein that is activated or upregulated during early stages of AD[114]. Significantly, PIN1 regulates the biological function and/or dephosphorylation of many mitotic MPM-2 antigens[33,44,60,68] and its depletion or inhibition induces mitotic catastrophe[8,33,58,70]. These results suggest that PIN1 may be neuroprotective against neurodegeneration in AD[5].

Such a notion has been supported by analyses of the distribution, modifications and genetic changes of PIN1 in normal and AD brains. Neurons in different subregions of the brain are known to have differential vulnerability to neurofibrillary degeneration in AD[115]. *PIN1* expression inversely correlates with the predicted neuronal vulnerability in normally aged brains and also with actual neurofibrillary degeneration in AD[59]. Proteomic approaches have confirmed that PIN1 can be downregulated in the brains of patients with AD and have also uncovered that PIN1 can be inactivated by oxidative modification, which occurs at early stages of AD[17,66,67]. Moreover, it has recently been shown that human chromosome 19p13.2 — where the PIN1 gene is located[116] — is an AD genetic locus[117] and, in some studies, PIN1 promoter polymorphisms are associated with reduced PIN1 levels in blood cells and with an increased risk for AD[118], although other studies have failed to find this genetic link[119,120]. Therefore, considerable evidence suggests that PIN1 may be downregulated or inactivated by various mechanisms in AD.

Insights from mouse models. Analysis of neuronal phenotypes in *Pin1*-knockout mice has provided the most direct evidence for a pivotal role of PIN1 in AD. *Pin1*-null mice develop progressive age-dependent neuropathy that is characterized by motor and behavioural deficits, hyperphosphorylated tau that adopts abnormal conformations (including one that is recognized by the monoclonal antibody TG3), tau filament formation and neuronal degeneration[59]. These phenotypes resemble tauopathy phenotypes that are present in AD and in many tau-related transgenic mice[121–125]. Recent studies have shown that PIN1 also contributes to Aβ-related pathologies[53,126]: one group reported that PIN1 does not bind to the pThr668-Pro motif in full-length APP, but rather binds to this motif only in the C-terminal C99 fragment of APP and reduces Aβ production from this fragment[126]. In contrast to this, Pastorino *et al.* have shown that PIN1 does bind and isomerizes the pThr668-Pro motif in full-length APP, thereby regulating the APP intracellular domain between two distinct conformations[53] (FIG. 2). Moreover, *PIN1* overexpression promotes non-amyloidogenic APP processing and reduces Aβ production, whereas *Pin1* knockout alone or in combination with APP mutant overexpression in mice increases amyloidogenic APP processing and selectively elevates insoluble Aβ42 in brain in an age-dependent manner; Aβ42 is localized to multivesicular bodies of neurons[53], as shown in human AD and APP transgenic mice before plaque pathology[127].

In contrast to most other AD mouse models in which the overexpression of one or several genes is used to induce certain AD phenotypes[103–107,128], *Pin1* is the only gene known so far that, when deleted in mice, can cause tau- and Aβ-related pathologies and neurodegeneration in an age-dependent manner, resembling many aspects of human AD[53,59]. These findings may provide evidence of a molecular link between tangles and plaques. Interestingly, a recent epidemiological study has found that the risk of developing cancer is less among participants with Alzheimer's type dementia versus participants who are non-demented, and that the risk of developing Alzheimer's type dementia may be less for participants with a history of cancer[129]. These findings are consistent with the opposite effects of PIN1 on the pathogenesis of cancer and AD, and the intimate connection between these two major age-dependent diseases[6].

A model for PIN1 in AD. Even though the aberrant regulation of APP and tau leads to different pathologies in AD, some underlying molecular defects might be similar [16,33,44,53,59] (FIG. 4). In the case of APP, phosphorylation of the Thr668-Pro motif has been proposed to be important in dictating the pathway of APP processing; *trans* protein is implemented in the non-amyloidogenic pathway, whereas the protein in the *cis* confirmation follows the amyloidogenic pathway[53]. APP is in *trans* before phosphorylation, but has a tendency to be in *cis* after phosphorylation due to local structural constraints[57]. Although the *cis* content is only ~10%, it may function as a 'buffer' population whereby, as mentioned earlier, PIN1 would rapidly

Activator protein-1
(AP1). A transcription factor that consists of either a Jun–Jun homodimer or a Jun–Fos heterodimer.

Senile plaques
One of the two neuropathological hallmarks found in the brains of patients with Alzheimer's disease. Plaques are primarily composed of Aβ peptides that are derived from amyloid precursor protein.

Neurofibrillary tangles
The other neuropathological hallmark found in the brains of patients with Alzheimer's disease. Tangles consist mainly of the microtubule-binding protein tau in a hyperphosphorylated state.

TG3
A phospho- and conformation-specific monoclonal antibody that recognizes the phosphorylated Thr231 in the tangle-specific conformation of tau observed in brains from patients with Alzheimer's disease.

Aβ42
42-amino-acid-long Aβ peptides that are derived from amyloid precursor protein after sequential cleavages by β- and γ-secretases.

Box 4 | **The pathology of Alzheimer's disease**

Alzheimer's disease (AD) is the most common cause of dementia in today's ageing population; its neuropathological hallmarks are extracellular senile plaques and intracellular neurofibrillary tangles. Significant progress has been made towards understanding the pathogenesis of AD, with two dominant theories focusing on overproduction and/or reduced clearance of Aβ peptides (which are derived from amyloid precursor protein (APP)) and hyperphosphorylation/dysfunction of the microtubule-binding protein tau[103–107]. APP is processed by the non-amyloidogenic pathway through α- and γ-secretases to produce neurotrophic αAPPs, or by the amyloidogenic pathway through β- and γ-secretases to produce neurotoxic Aβ peptides. Mutations of proteins that regulate Aβ production have been well documented in AD, and transgenic mice that overexpress mutant APP or presenilins overproduce Aβ and develop plaques[103–107,128]. Although no tau mutation has been found in AD, tau mutations have been identified in frontotemporal dementia (FTDP-17), and transgenic mice that overexpress tau, especially its FTDP-17 mutants, exhibit age-dependent tauopathy phenotypes[103–107,128]. Moreover, Aβ can exacerbate tangle formation in tau-mutant mice[123,124], indicating the interactions between Aβ deposits and tau tangles. However, the molecular link(s) between these pathological hallmarks remain elusive. A growing body of evidence indicates some common features between degenerated AD neurons and the normal cell cycle, especially Pro-directed mitotic phosphorylation on certain proteins such as tau and APP[5,108,109], which points to a role for the phosphorylation-specific prolyl isomerase PIN1 in AD.

re-establish equilibrium if the *trans* (or *cis*) population was suddenly depleted. Therefore, if the *trans* pThr668-APP was rapidly depleted by the non-amyloidogenic pathway (FIG. 4), PIN1-catalysed prolyl isomerization might prevent an increase in amyloidogenic APP processing by catalysing *cis* to *trans* isomerization and preventing the build-up of *cis*[53]. However, during the development of AD, PIN1 function is downregulated and/or inhibited by oxidation or possible genetic alterations as discussed above, and/or APP phosphorylation is increased owing to various environmental or genetic factors[4,103–107]. Under these conditions, a higher concentration of the *cis* pThr668-Pro motif would be present for a longer time, which might promote amyloidogenic APP processing and Aβ overproduction, which in turn might also induce oxidative stress to further inactivate PIN1 (REF. 53) (FIG. 4).

In the case of tau, PIN1 promotes dephosphorylation of the pThr231-Pro motif by the *trans*-specific protein phosphatase PP2A. *Pin1* knockout causes accumulation of the pThr231-Pro motif in the tangle-specific conformation that is recognized by TG3 (TG3 probably recognizes a *cis* confirmation of tau)[33,59,130]. Therefore, PIN1 promotes tau dephosphorylation and restores tau function possibly by catalysing *cis* to *trans* isomerization (FIG. 4), although the structure of pThr231 that would help to confirm this is not available. When PIN1 activity drops below a threshold level, the *cis* pThr231-Pro motif accumulates, leading to tau hyperphosphorylation, aggregation and tangle formation (FIG. 4). Therefore, *cis* and *trans* conformations of the pThr-Pro motif in tau and APP are distinct not only in their structures, but also in their biological function or pathological consequences. By acting on this unique conformational switch, PIN1 is pivotal in protecting against age-dependent neurodegeneration in AD and might be a new drug target for treating the disease (BOX 3).

PIN1, immune responses and microbial infection

The importance of NF-κB in oncogenesis[131] led to an investigation of the relationship between *PIN1* overexpression and NF-κB activation in breast cancer, which has identified a novel role of PIN1 in NF-κB signalling[48]. In response to cytokine signals, PIN1 binds to and isomerizes the phosphorylated p65 subunit of NF-κB and prevents NF-κB from binding to and thereby being inhibited by the endogenous inhibitory protein IκB (inhibitor of NF-κB), which leads to increased NF-κB activity. Furthermore, PIN1 inhibits ubiquitin-mediated proteolysis of p65 by SOCS1 (suppressor of cytokine signalling-1)[48]. Importantly, PIN1-deficient mice and cells are refractory to NF-κB activation by cytokine signals. *PIN1* overexpression in cancer cells would, therefore, uncouple the inhibition of NF-κB by IκB, which might explain why NF-κB signalling is overactivated in spite of IκB upregulation in many cancer cells[48]. PIN1 has also been shown to activate the transcription factor AP1 (REFS 45,132). Given the central role of NF-κB and AP1 in immune responses[133,134], it would be interesting to examine the significance of PIN1 in immunity.

Recent studies have shown that PIN1 has an important role in asthma development and in the response to microbial infection[135–137]. During asthma, PIN1 is activated by dephosphorylation and functions as an essential component of the ribonucleoprotein complex, which is responsible for GM-CSF (granulocyte-macrophage colony-stimulating factor) mRNA stabilization, cytokine secretion and eosinophil survival. This suggests an important role for PIN1 in the development of asthma; thus, inhibiting its function offers a possible treatment[135].

In addition, PIN1 may be involved in innate immunity against viral infection. Stimulation of TLR3 (Toll-like receptor-3) by double-stranded RNA leads to the formation of a multisubunit complex that includes IRF3 (interferon-regulatory factor-3). This complex regulates the expression of interferon-β (IFNβ), which controls the transcription of several hundred genes that lead to a cellular antiviral state that prevents viral replication. However, the production of IFNβ is tightly regulated to prevent tissue damage. PIN1 binds to phosphorylated IRF3 and promotes its ubiquitin-mediated proteolysis[136]. *PIN1* knockdown or knockout results in sustained IRF3-dependent activation and enhanced IFN-β production[136].

PIN1 as a molecular timer

Given that PIN1 participates in diverse cellular processes, the question arises as to whether there is an underlying theme and why the cell would employ such an additional regulatory mechanism after phosphorylation. The answer probably lies in the unique structure and crucial regulatory role of certain pSer/Thr-Pro motifs. It has become clear that kinases and phosphatases that modify these motifs, as well as proteins that interact with these motifs (for example F-box proteins targeting phosphorylated proteins for degradation), are conformation-specific[31–33,138].

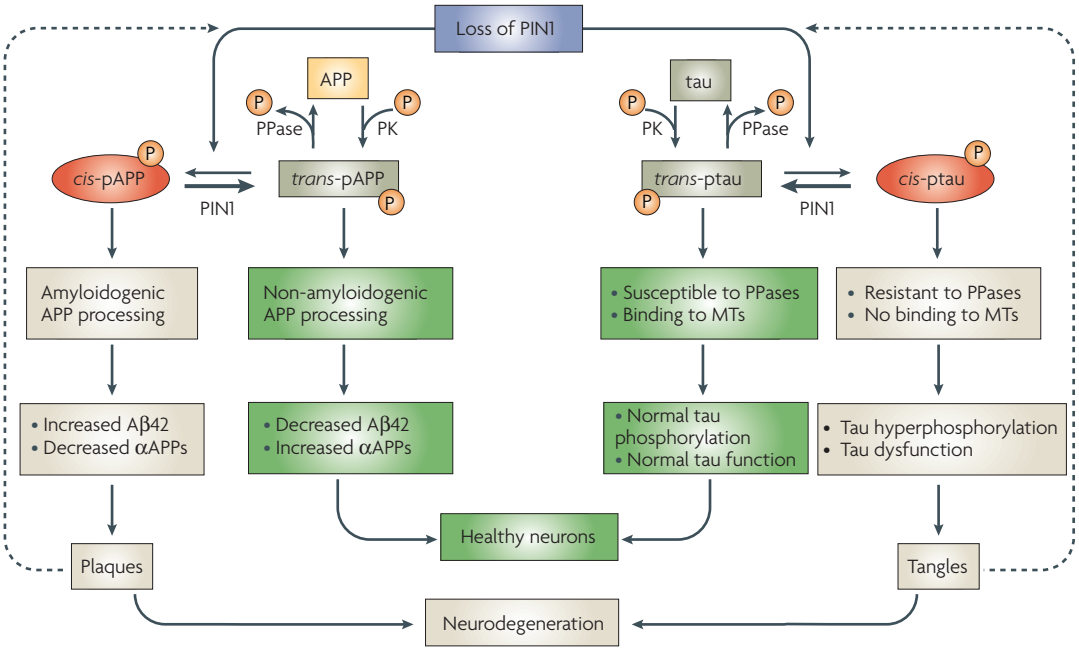


Figure 4 | **The regulation of tau function and APP processing by PIN1 in healthy and Alzheimer's neurons.** Both tau and amyloid precursor protein (APP) may be phosphorylated by protein kinases (PKs) as part of their normal function. The *trans*-conformation of phosphorylated (p) tau and APP may represent the physiological conformation that promotes their normal function (green boxes). PIN1 (protein interacting with NIMA (never in mitosis A)-1) expression is induced during neuron differentiation, and may be needed to maintain normal neuronal function by preventing the unscheduled activation of mitotic events and/or controlling the function of phosphoproteins in the event that they become abnormally phosphorylated. For example, by catalysing isomerization of the *cis* to *trans* conformation, PIN1 might promote non-amyloidogenic APP processing and reduce Aβ production, as well as promote tau dephosphorylation and restore tau function. However in Alzheimer's disease (AD), a loss of PIN1 function, either through downregulation of PIN1 function, oxidative inactivation, phosphorylation or possible genetic changes, can lead to a build-up of *cis*-pSer/Thr-Pro motifs. *Cis*-ptau and *cis*-pAPP are proposed to represent pathological conformations (red ovals). *Cis*-pAPP is processed by the amyloidogenic pathway, which leads to a build-up of amyloid β-42 (Aβ42), decreased levels of neurotropic αAPPs and the resultant formation of amyloid plaques. *Cis*-pTau is resistant to protein phosphatases (PPases), which leads to a loss of microtubule (MT) binding, hyperphosphorylated tau and the formation of neurofibrillary tangles. The formation of tangles and plaques might further reduce PIN1 function by sequestering PIN1 and inducing PIN1 oxidative modifications, respectively, in a positive feedback loop. In addition, a lack of proper PIN1 function may lead to further activation of mitotic kinases, which may further increase the phosphorylation of tau, APP and other proteins, and eventually cause neuronal death. Therefore, PIN1 deregulation might act on multiple pathways to contribute to AD development.

When the *cis* or *trans* isomer is depleted in the cell (for example, by isomer-specific degradation, dephosphorylation or interactions), restoration of the equilibrium between *cis* and *trans* isomers would require many minutes without a PPIase. Although PIN1 cannot shift the *cis*/*trans* equilibrium of substrates, it can maintain equilibrium populations of targeted *cis* and *trans* substrate conformations on the millisecond timescale, which is more relevant for the regulation of dynamic biological processes.

On the cellular level, a model for PIN1 function will probably depend on the cell type and the specific signalling cascades involved. It is crucial to distinguish between PIN1 function in normal cells, where PIN1 levels and post-translational modifications are tightly regulated[58,63], and its function in pathological conditions, where these PIN1 regulatory mechanisms are constitutively altered[45,46]. For example, because the function of PIN1 fluctuates during the cell cycle in normal cells, it is conceivable that PIN1 participates in maintaining the balance between and coordinating the timing of pro-proliferative and pro-apoptotic signals, as well as in coordinating progression through the cell cycle. However, this delicate balance is disrupted in cancer cells. Oncogenic activation will trigger signalling pathways that lead to increased Pro-directed phosphorylation and constitutive *PIN1* overexpression by E2F in a positive feedback loop. This effect accentuates the resulting imbalance and thereby enhances these signalling events. In this scenario, PIN1 enhances downstream signalling by amplification of positive feedback mechanisms and suppression of negative feedback mechanisms, which contribute to tumorigenesis (FIG. 3).

Conclusions and future directions

The discovery of the phosphorylation-specific PPIase PIN1 has established conformational regulation after phosphorylation as a new signalling mechanism. Such conformational regulation can have a profound impact on many key proteins in diverse cellular processes.

PIN1 has emerged as a new molecular timer to coordinate multiple pathways synergistically in one direction under certain conditions. Importantly, PIN1 function is tightly regulated under physiological conditions and its deregulation has a pivotal role in a growing number of pathological conditions; thus, it is a potential new diagnostic and therapeutic target.

Compared with kinases and phosphatases, PIN1 is a much newer player in phosphorylation signalling, and it is even more difficult to visualize PIN1-induced conformational changes after phosphorylation. A major challenge for the future will be to define structural and functional differences of these *cis* and *trans* phosphorylated proteins, to develop tools to visualize PIN1-catalysed conformational changes, to determine how PIN1 controls these conformations and to correlate these different conformations with protein function during cell signalling. Another challenge will be to determine PIN1 upstream regulatory pathways, to identify all pathological consequences that are associated with PIN1 deregulation and to elucidate their underlying mechanisms. Given that deregulated kinases are well-established drug targets, it would be beneficial to develop drugs that target PIN1 to examine whether this new mechanism can be used for treating certain human diseases. Finally, there are multiple PIN1 homologues in plants[42], two in *Drosophila* and also possibly in mice[43], and phosphorylation-specific PPIases can be detected in PIN1-deficient cells. This may open up the opportunity for PIN1-like enzymes that are yet to be discovered.

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DATABASES
OMIM: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM
Alzheimer's disease | frontotemporal dementia
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APP | CDC25C | p300 | PIN1 | PLK1 | Shc | WEE1

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