Published in final edited form as:

Trends Biochem Sci. 2011 October ; 36(10): 501–514. doi:10.1016/j.tibs.2011.07.001.

The prolyl isomerase Pin1 as a molecular switch to determine the fate of phosphoproteins

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Abstract

Pin1 is a highly conserved enzyme that only isomerizes specific phosphorylated Ser/Thr-Pro bonds in certain proteins, thereby inducing conformational changes. Such conformational changes represent a novel and tightly controlled signaling mechanism regulating a spectrum of protein activities in physiology and disease, often through phosphorylation-dependent, ubiquitin-mediated proteasomal degradation. In this review, we summarize recent advances in elucidating the role and regulation of Pin1 in controlling protein stability. We also propose a mechanism by which Pin1 functions as a molecular switch to control the fates of phosphoproteins. We finally stress the need to develop tools to directly visualize Pin1-catalyzed protein conformational changes as a way to determine their roles in the development and treatment of human diseases.

Characterization of Pin1 function

One of the most important and universal regulatory mechanisms in the cell is the reversible phosphorylation of proteins [1, 2]. Therefore, the ability to define the regulatory components of the phosphorylation/dephosphorylation cascades and the interactions between these components with other cellular components are critical to our understanding of diverse biological processes and the molecular mechanisms underlying various human diseases for efficacious and rational drug design. Indeed, the reversible phosphorylation on certain serine or threonine residues preceding a proline (pSer/Thr-Pro) represents a key switch for controlling the function of many signaling molecules in various cellular processes. Notably, recent studies have shown that Ser/Thr-Pro phosphorylated proteins are subjected to post-phosphorylation conformational modifications by the phosphorylation-specific peptidyl-prolyl *cis/trans* isomerase (PPIase) Pin1 [3, 4].

Pin1 is a relatively small enzyme [5] that contains an N-terminal WW domain that acts as a phosphoprotein-binding module [6] and a C-terminal catalytic domain that is distinct from other conventional PPIases [7, 8]. Owing to its unique WW and catalytic domains, Pin1 isomerizes specific phosphorylated Ser/Thr-Pro bonds, and regulates the function of a

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defined subset of phosphoproteins [8]. Isomerization of Ser/Thr-Pro motifs is especially important because kinases and phosphatases specifically recognize the *cis* or *trans* conformation of the prolyl peptide bond of their substrates [9, 10] and phosphorylation further slows down the isomerization rate of proline [4, 8]. Pin1 activity controls a subset of protein functions in diverse cellular processes such as cell cycle and cell growth [3, 4, 10–19]. Importantly, Pin1 is tightly regulated by several mechanisms [17, 20–23] and its deregulation can contribute to an increasing number of human diseases including aging, cancer, neurological disorders, autoimmune and inflammatory diseases [4, 11, 24].

Pin1-mediated post-phosphorylation regulation can have profound effects on phosphorylation-dependent signaling by regulating a spectrum of target activities, with changes in protein stability being the most common consequence. Since the discovery of ubiquitin-mediated protein degradation nearly 30 years ago [25], the ubiquitin-proteasome system (UPS) has been found to be responsible for the regulated degradation of the majority of intracellular proteins through the 26S proteasome [26]. The small 76 amino acid protein ubiquitin exerts a wide range of effects by selectively conjugating with the target protein via a process called ubiquitylation [27]. Notably, phosphorylation of Ser/Thr-Pro motifs constitutes a major regulatory mechanism in controlling ubiquitin-mediated proteolysis. For example, the SCF (Skp1-Cullin-F-box) complex directs phosphoproteins to be recruited for ubiquitylation-mediated degradation [28]. Recent structural studies indicate that F-box proteins preferentially bind substrates via a *trans* conformation of the phospho-Ser/Thr-Pro motif [29], suggesting that the *cis* or *trans* conformation of proline could be a critical determinant in regulating protein degradation.

Extensive discussions on Pin1 in general and its specific roles in cellular processes related to diseases have been covered in several reviews [3, 4, 30–33]. In this review, we will focus on discussing how Pin1-mediated prolyl *cis/trans* isomerization can influence the stability of its substrates, particularly via phosphorylation-dependent ubiquitin-mediated degradation, under physiological and pathological conditions (Table 1)..

Pin1 regulates the stability of cell cycle proteins

Pin1 interacts with and regulates the stability of its phospho-substrates, thereby providing a unique post-phosphorylation regulatory mechanism for biological processes. By acting on multiple targets, Pin1 often synergistically drives particular cellular processes in one direction. For example, Pin1 activates or stabilizes numerous oncoproteins and growth enhancers and also inactivates or destabilizes a number of tumor suppressors and growth inhibitors to promote tumorigenesis.

Pin1 enhances protein stability

Pin1 protein is overexpressed in many human cancers, and this overexpression is associated with poor clinical outcome [17, 34]. Indeed, by upregulating the expression of oncoproteins and disrupting cell cycle progression, Pin1 can promote oncogenesis. For instance, Pin1 regulates the expression of cyclin D1 by cooperating with oncogenic Ras signaling and inhibits the interaction of β -catenin with the tumor suppressor APC, thus directly stabilizing cyclin D1 [22]. Overexpression of cyclin D1 contributes to cell transformation and is found in about half of breast cancer patients [35]. Aberrant activation of β -catenin results in enhanced transcription of cyclin D1, which leads to oncogenesis [36]. In cell culture studies, Pin1 regulates the stability and subcellular localization of β -catenin [14]. Increased β -catenin expression correlates with upregulated Pin1 expression in breast tumors while β -catenin levels are decreased in Pin1 knockout mouse tissues [14]. Pin1 binds and isomerizes the phosphorylated Ser246-Pro motif in β -catenin and thus inhibits the interaction of β -catenin with APC, which exports nuclear β -catenin into the cytoplasm for degradation. The aberrant

accumulation of β -catenin in the nucleus results in enhanced transactivation of downstream oncogenes such as c-Myc and cyclin D1.

Loss of Pin1 in mice displays a range of cell proliferative phenotypes, including reduced body weight, testicular atrophy and retinal degeneration, which strikingly resembles cyclin D1 knockout mice [11]. More importantly, cyclin D1 protein levels are significantly decreased in tissues displaying severe phenotypes. Indeed, Pin1 stabilizes cyclin D1 where the half-life of cyclin D1 protein decreases at a higher rate in *Pin1*-knockout mouse embryonic fibroblasts (MEFs) compared wild-type MEFs [11]. At the molecular level, the kinase GSK-3 β phosphorylates the Thr286-Pro motif of cyclin D1 to allow binding to CRM1, which controls cyclin D1 turnover and localization by shuttling nuclear cyclin D1 to the cytoplasm leading to degradation [37, 38]. Pin1 binds to cyclin D1 at the phosphorylated Thr286-Pro motif and presumably catalyzes its isomerization which further inhibits its interaction with CRM1, resulting in stabilization and an accumulation of cyclin D1 in the nucleus [11]. Therefore, Pin1 plays a dual role in regulating cyclin D1 levels both at the transcriptional as well as the post-translational level.

The signaling cascade triggered by Ras activates c-Jun N-terminal kinases (JNKs) to phosphorylate c-Jun on Ser63 and Ser73 sites and enhances transactivation of c-Jun target genes, such as cyclin D1 [39]. After Pro-directed phosphorylation by JNK, the activity of c-Jun is further regulated by Pin1, which binds to phosphorylated Ser63/73-Pro motifs in c-Jun, resulting in an increased transcriptional activity of c-Jun towards the cyclin D1 promoter [17]. Pin1 increases the protein stability of c-Jun by inhibiting c-Jun polyubiquitylation, which blocks granulocyte differentiation mediated by the transcription factor CCAAT enhancer binding protein α (C/EBP α) [40]. Upregulation of Pin1 in cancer cooperates with Ras signaling, resulting in increasing c-Jun-mediated cyclin D1 transactivation. Therefore, aberrant upregulation of Pin1 also contributes to myeloid leukemia development through stabilizing c-Jun and inhibiting granulocyte differentiation mediated by C/EBP α function.

Increased expression of the antiapoptotic protein, myeloid cell leukemia-1 (Mcl-1), is associated with cell immortalization and chemoresistance in human malignancies [41]. Phosphorylation of Mcl-1 by GSK-3 β can recruit the E3 ligase, β -Trcp, for Mcl-1 degradation which leads to cellular apoptosis, tumor suppression and chemosensitization [42]. The expression of Mcl-1 and Pin1 is positively correlated. Furthermore, elevated expression of these two proteins is associated with a poor survival rate in human breast cancer [43]. Importantly, Pin1 is identified as the novel regulator of ERK-induced Mcl-1 upregulation. The phosphorylation of Thr92-Pro and Thr163-Pro motifs of Mcl-1 by ERK induces the interaction of Pin1 and further stabilizes Mcl-1 protein [43]. Taken together these studies unravel a novel mechanism which links the ERK/Pin1 pathway and Mcl-1 mediated chemoresistance.

Pin1 decreases protein stability

In addition to stabilizing and activating oncoproteins, Pin1 can also inactivate and destabilize a large number of tumor suppressors and growth inhibitors including PML, Daxx, RAR α , SMRT, Smad, and TRF1 (Table 1). Although Pin1 inactivates a few oncoproteins such as c-Myc, these results were mainly performed using *in vitro* models. In most cases, Pin1 appears to play a pivotal role in destabilizing tumor suppressors, lending further support to the idea that Pin1 could be a potential target for therapeutic cancer drugs [44, 45].

Constitutive overexpression of c-Myc is associated with tumorigenesis while ectopic expression of c-Myc in cultured cells induces neoplasia. Dynamic levels of c-Myc, which is

highly regulated through phosphorylation at Ser62 and Thr58 by ERK and GSK3 β , respectively, is essential for normal cell proliferation [33]. The phosphorylation of Ser62 stabilizes c-Myc; conversely, subsequent Thr58 phosphorylation results in c-Myc degradation via the ubiquitin-proteasome pathway. Furthermore, the phosphorylation of Thr58 promotes the dephosphorylation of Ser62, which is important for ubiquitin-dependent degradation of c-Myc [40]. Pin1 specifically isomerizes the *cis/trans* conformation of phosphorylated Thr58-Pro which in turn enhances the dephosphorylation activity of PP2A on Ser62. In the absence of Pin1, mutation of Thr58 or inhibition of PP2A prevents dephosphorylation of c-Myc and thus polyubiquitylation, resulting in aberrant stabilization and accumulation of c-Myc protein in a primary human cell transformation assay [46]. Additionally, a recent study showed that the tumour suppressor scaffold protein, Axin1, facilitates the formation of a c-Myc degradation complex, which consists of GSK-3 β , Pin1, and PP2A. However, depletion of Axin1 disrupted the interaction of Axin1 with the degradation complex, subsequently causing a reduction of Thr58 phosphorylation and an enhancement of Ser62 phosphorylation, thus increasing the stability of c-Myc [47]. These studies provide evidence that these proteins including GSK-3 β , Pin1, and PP2A are within the required proximity to interact and regulate the stability of c-Myc protein.

Similar to c-Myc, misregulation of cyclin E function also causes cell cycle defects and the onset of oncogenesis [33]. Cyclin E is phosphorylated by Cdk2 and GSK-3 β at Ser380 and Ser384, respectively [48]. The phosphorylation of Ser384 on cyclin E allows Pin1 to bind and regulate its protein turnover. Pin1 promotes cyclin E protein degradation during cell cycle progression, whereas the absence of Pin1 results in an accumulation of cyclin E at the G₁/S phase of the cell cycle [48]. These studies demonstrated that a decreased Pin1 level results in a selective growth disadvantage due to delayed G₀/G₁-S phase progression.

Retinoic acid receptor (RAR) mediates the physiological activity of *all-trans* retinoic acid in myeloid cells [49]. The promyelocytic leukemia protein (PML) plays diverse roles in different cellular processes including cell cycle progression and is involved in acute promyelocytic leukemia (APL), a type of acute myelogenous leukemia (AML), by facilitating PML-RAR α oncoprotein expression [49]. Pin1 is overexpressed in AML and its interaction with RAR α and PML-RAR α is recently reported [50]; the overexpression of Pin1 inhibits ligand-dependent activation of RAR α and PML-RAR α transcriptional activity. Pin1 functionally inhibits RAR α and PML-RAR α through degradation of the nuclear receptors via the proteasome-dependent pathway. Inhibition of Pin1 in AML cells stabilizes RAR α and PML-RAR α [50]. Notably, the phosphorylation of Ser77 in RAR α is essential for Pin1 interaction and a point mutation of Ser77 to Ala abrogates RAR α degradation [51]. Therefore, Pin1 regulates the RAR α protein stability.

In addition to regulating PML-RAR α protein stability [50], the protein stability of PML is also regulated via interaction with Pin1 through four phosphorylated Ser-Pro motifs at the C terminus of PML [44]. The interaction with Pin1 leads to PML degradation in a phosphorylation dependent manner. Interestingly, the sumoylation of PML blocks this interaction by preventing Pin1-mediated PML degradation. Pin1-mediated PML degradation protects cancer cells from H₂O₂-induced cell death and increases the rate of cell proliferation [44]. In addition, Pin1 and PML interaction is mediated by H₂O₂ and insulin growth factor-1 (IGF-1) which upregulate and downregulate PML expression, respectively [52].

Pin1 regulates a variety of oncoproteins associated with transcription by increasing β -catenin and nuclear factor-kappa B (NF- κ B) stability, and decreasing the stability of c-Myc, cyclin E and RAR. Other transcription associated proteins including the steroid receptor coactivator-3 SRC-3/AIB-1, which is an oncogene that is overexpressed in breast and ovarian cancers

[53], were found to interact with Pin1; the primary role of Pin1 is also to regulate protein stability in this situation. Extracellular signals including steroid hormones, growth factors, and cytokines induce SRC-3 phosphorylation, facilitating its interaction with CREB binding protein (CBP)/E1A binding protein p300 (p300) and nuclear receptors and triggering its oncogenic activity. Pin1 acts as a co-activator of steroid receptor through regulating the protein turnover of SRC-3 [53]. Initially, Pin1 binds to phosphorylated SRC-3 and enhances its interaction with CBP/p300, thus increasing the transcription of downstream target genes. Then Pin1 subsequently promotes SRC-3 degradation. Therefore, Pin1 has a dual function; while it acts as a transcriptional coactivator of SRC-3 to promote SRC-3 activation in breast cancer, it also regulates the protein turnover of SRC-3 so that SRC-3 can properly respond to new environmental signals.

Transforming growth factor- β (TGF- β) signaling is essential in regulating proliferation, differentiation, migration and apoptosis [54]. Intracellular Smad proteins mediate TGF- β signaling and are regulated by the ubiquitin-proteasome system via E3 ubiquitin ligase family, Smad ubiquitin regulatory factor 1 and 2 (Smurf1/2) and a Smurf1-related protein [55, 56]. Phosphorylation of Smad1 in the linker region promotes an interaction to Smurf1, which results in polyubiquitylation of Smad1 [57]. Pin1 binds Smad2/3 and negatively regulates TGF- β signaling by downregulating Smad2/3 protein levels [58]. Specifically, conformational changes induced by Pin1 in the linker region (potential sites include phosphorylated Thr179, Ser204, Ser208, Ser213) reduce Smad2/3 protein levels by promoting the interaction of Smurf2 with Smad, leading to enhanced Smad ubiquitylation. Thus, aberrant expression of Pin1 in cancer cells could adversely affect TGF- β signaling thus providing new insights into the mechanisms of impaired TGF- β signaling in cancers.

The control of the turnover of protein kinases in cell cycle progression is vital in regulating cell proliferation [59]. Deregulation of the protein levels of G protein-coupled receptor kinase 2 (GRK2), a key player in G-protein coupled receptor regulation, impacts cell proliferation and cancer formation [60]. In particular, GRK2 was reported to regulate TGF-mediated cell growth arrest and apoptosis, thyroid stimulating hormone, and platelet-derived growth factor (PDGF)-dependent proliferation in various cell types [61, 62]. During G₂/M transition, CDK2 phosphorylates GRK2 at the Ser670 residue leading to transient downregulation of GRK2 protein levels. Pin1 binds phosphorylated Ser670 of GRK2 and triggers GRK2 degradation [63]. Notably, abnormal accumulation of GRK2 delays cell cycle progression. Also, the stabilization of GRK2 inversely correlates with p53-mediated response and apoptosis induction in the presence of DNA damaging agents [63], suggesting that GRK2 potentiates cell cycle arrest protraction and cell survival in the G₂/M checkpoint. Therefore, GRK2 turnover is regulated by Pin1, which affects cell cycle progression independent of the receptor. Together, this study reveals a novel role for GRK2 in cell proliferation.

Pin1 regulates protein stability in neurodegeneration

Unlike the most differentiated cells in the body, where Pin1 expression is greatly reduced, Pin1 expression is induced during neuronal differentiation and exists in neurons at considerable high levels [10, 12, 24]. Recent studies have indicated that Pin1 regulates several neuronal substrates, notably tau and amyloid precursor protein (APP), and plays an important role in age-dependent neurodegeneration. However, the role of Pin1 in healthy neurons and during development of the nervous system remains largely unknown.

Pin1 negatively regulates proteins associated with Alzheimer's disease

The presence of neurofibrillary tangles and neuritic plaques in the brain are neuropathological hallmarks of Alzheimer's disease (AD). The neurofibrillary tangles

contain paired helical filaments composed of hyperphosphorylated microtubule-associated protein, tau, which is unable to bind microtubules and promote their assembly. Conversely, the neuritic plaques arise due to overproduction and/or lack of clearance of amyloid- β peptides (A β) derived from APP [64, 65]. Phosphorylation of tau and APP on Thr668-Pro residues regulates the formation of neurofibrillary tangles and amyloid- β peptides *in vitro* [66, 67]. Although Pin1 is expressed in most human neurons, its levels are abnormally low in AD brains. To date, *PIN1* is the only known gene whose knockout is sufficient to trigger tau- and A β -related pathologies and neuronal degeneration in an age-dependent manner, resembling many aspects of human AD [10, 12, 13, 68, 69].

Pin1 activity can directly restore the conformation and function of phosphorylated tau by indirectly promoting its dephosphorylation [13]. Specifically, Pin1 acts on the phosphorylated Thr231-Pro in tau to promote PP2A-mediated dephosphorylation and restore its microtubule binding function [10, 13, 67]. Moreover, whereas Pin1 promotes the degradation of wild-type tau, the loss of *PIN1* induces the accumulation of tau and thus enhances its possibility to be hyperphosphorylated [70], which might also provide an explanation for how the loss of Pin1 in mice causes markedly increased levels of hyperphosphorylated tau. In addition, Pin1 also regulates APP processing and A β production [64]. In particular, Pin1 interacts with the phosphorylated Thr668-Pro motif of APP and profoundly accelerates its intracellular domain isomerization; however, whether this conformational change regulates APP stability is unknown. In mouse brain, the absence of Pin1 increases amyloidogenic APP processing and elevates the toxic species of A β 42 in an age-dependent manner [68]. Although deregulation of Pin1 might affect the conformations of phosphorylated Thr-Pro motifs in tau and APP in a similar manner, leading to a similar outcome, the resulting pathological changes could indeed be distinct [10, 12, 13, 68].

Pin1 stabilizes proteins associated with Parkinson's disease

Parkinson disease (PD) is characterized by the loss of dopaminergic neurons in the substantia nigra and the formation of Lewy bodies, cytoplasmic inclusions containing α -synuclein aggregates, in surviving neurons [71]. Pin1 accumulates in Lewy bodies and enhances the formation of α -synuclein inclusions by protecting α -synuclein from degradation [72]. However, α -synuclein degradation is not directly linked to Pin1 PPIase activity given that Pin1 and α -synuclein do not directly interact. Instead, Pin1 binds synphilin-1, an α -synuclein regulatory protein, at phosphorylated Ser211-Pro and Ser215-Pro motifs and enhances its interaction with α -synuclein, thereby facilitating the formation of α -synuclein inclusions [72]. Therefore, Pin1-mediated prolyl-isomerization modulates α -synuclein aggregation indirectly by acting on its regulatory protein, synphilin-1, in the resultant Lewy body formations in PD, lending further support for its role in maintaining normal neuronal function. However, the mechanism underlying the role of Pin1 in PD remains largely unknown.

Pin1 regulates protein stability in telomere regulation and aging

Telomeres are pivotal in maintaining cellular proliferative capacity and genomic stability; by contrast, telomere loss has been implicated in aging [73, 74]. A key regulator in telomere maintenance is the telomeric DNA-binding protein TRF1, which negatively regulates telomere elongation without affecting telomerase activity [75–77]. TRF1 was also identified as Pin2 in the same combined genetic screen for Pin1 [5, 78], but little is known about whether TRF1 is regulated by upstream signals. Recent studies indicate that TRF1 is a major substrate for Pin1 in telomere regulation and aging [79]. Pin1 interacts with the conserved phosphorylated Thr149-Pro motif in TRF1. Furthermore, Pin1 inhibition renders TRF1 almost completely resistant to degradation and enhances TRF1 binding on telomeres, leading to gradual telomere shortening through a TRF1-dependent mechanism. Moreover,

Pin1^{-/-} mice display elevated TRF1 levels, telomere loss and a range of premature aging phenotypes; intriguingly, within one generation, these phenotypes are similar to those in telomerase knockout mice after 4–5 generations. These results demonstrate the pivotal role of Pin1 in regulating the role of TRF1 in telomere maintenance and ageing. Notably, telomere has been shown to be inversely correlated with AD; diseased neurons contain shorter telomeres than healthy controls [80]. These results suggest that Pin1-dependent regulation of telomere maintenance might also contribute to the development of AD.

Molecular mechanisms of Pin1-dependent regulation of protein stability

Phosphorylation of Ser/Thr-Pro motifs further restrains the already slow *cis/trans* prolyl isomerization of peptide bonds [8], and renders them resistant to the catalytic action of conventional PPIases, including cyclophilins and FK506-binding proteins [8]. Pin1 is a representative member of a subfamily of phosphorylation-specific PPIases that isomerizes only phosphorylated Ser/Thr-Pro bonds [8]. Although Pin1 is known to regulate substrate stability and function by acting on specific phosphorylated Ser/Thr-Pro motifs (Table 1), the central underlying molecular mechanism remains elusive. Here, we discuss several plausible molecular mechanisms (Figure 1).

We propose that the conformational changes induced by Pin1 on the wide range of substrates, e.g. c-Myc [46], cyclin E [48], SRC-3 [53], Smad [58] and Che-1 [81] boost the *cis/trans* isomerization rate thereby establishing the correct conformation for subsequent ubiquitylation of phospho-proteins by an E3 ligase and subsequent proteasome-mediated protein degradation. For example, Pin1 interacts with Che-1, a human RNA polymerase II-binding protein involved in p53 transcription and in the maintenance of G₂/M checkpoint upon apoptotic DNA damage, at a phosphorylated Thr144-Pro motif and induces its conformational change. This conformational change enhances the interaction between Che-1 and its E3 ligase, HDM2. Che-1 is then subjected to rapid ubiquitin-mediated protein degradation [81]. By contrast, Pin1-catalyzed prolyl *cis/trans* isomerization of phosphorylated substrates could render the protein unfavorable for ubiquitin ligase binding or inhibit ubiquitin ligase access to the phosphorylated sites to increase the half-life of the protein. Thus, we suggest that another group of Pin1 substrates, e.g. cyclin D1 [11], β -catenin [14], NF- κ B [15], p53 (Box 1) [16], p73 [82] and Tax (a viral transcription protein) [83] are subjected to Pin1-induced catalytic activity to block ubiquitin-mediated protein degradation and enhance protein stability. As an example, co-expression of Pin1 and the putative ubiquitin ligase, SOCS-1, significantly blocks the ubiquitin-mediated degradation of p65. Pin1 induces the *cis/trans* conformational change of phosphorylated p65 into an unfavorable conformation for SOCS-1 recognition. The specific Ser/Thr-Pro motifs in these two groups of Pin1 substrates might have different preferences to be in a *cis* or *trans* isomer after phosphorylation, probably due to local structural constraints. Thus, Pin1 might, in principle, convert a *cis* isomer to a *trans* isomer, or from a *trans* isomer to a *cis* isomer depending on the specific phosphorylated Ser/Thr-Pro motifs as a way to regulate protein stability.

Box 1

Pin1 regulates p53 protein stability in genotoxic stress and apoptosis
The tumor suppressor p53 plays a key role in the DNA damage-induced checkpoint pathway. p53 transcriptional activity is regulated by post-translational modifications, and associations with its regulatory proteins determine whether a cell undergoes cell cycle arrest or apoptosis in response to oncogenic stimuli [18, 19]. In particular, DNA damage induces the phosphorylation of p53 at multiple Ser/Thr-Pro residues (Ser33, Ser46, Thr81 and Ser315 have been reported), thereby enhancing its interaction with Pin1 [16, 18, 19].

The Pin1 WW domain is required for this interaction, and the PPIase activity drives p53 conformational changes resulting in enhanced p53 stability and transactivation of its target genes, thereby promoting cell cycle arrest and apoptosis [16, 18, 19]. In addition, Pin1 might mediate p53 stability by interrupting the interaction with its E3 ligase Mdm2. For example, the interaction between Pin1 and the p53 Thr81-Pro motif drives Chk2-mediated phosphorylation of p53 at Ser20 [139] and induces p53 dissociation from Mdm2 [18]. Moreover, the inhibition of Pin1 function by an organic compound Juglone (5-hydroxynaphthoquinone) results in an accumulation polyubiquitylated p53, thereby leading to decreased cellular p53 levels [105]. In addition, although Mdm2 can effectively induce p53 polyubiquitylation, Pin1 can significantly inhibit this Mdm2-dependent polyubiquitylation; this finding suggests that Pin1 and Mdm2 might antagonize each other's function and thus play an opposite role in controlling the fate of p53 [105]. Another line of direct evidence for the physiological relationship between Pin1 and p53 was provided by the study of *Pin1^{-/-} Trp53^{-/-} double knockout mice* [140]. Unlike *Trp53^{-/-} mice*, *Pin1^{-/-} Trp53^{-/-} double knockout mice* are free of malignant tumors. Further studies showed that the Pin1 proline-rich domain (PRD) might regulate p53 stability; however, it is not essential for p53 activation and tumor suppression [141].

As mentioned above, the *cis/trans* isomerization of phosphorylated Ser/Thr-Pro peptide bond occurs very slowly in nature; whereas phosphorylation and subsequent attachment of ubiquitin on target proteins, by contrast, occur rapidly under normal conditions. The mechanism for ubiquitylation of phosphorylated proteins might highly depend on a correct *cis/trans* protein conformation, and thus the isomerization of a phosphorylated protein that is destined for ubiquitylation could be a rate-determining step in the phosphorylation-dependent ubiquitin-mediated proteasome degradation pathway. Although Pin1 is not a component of the ubiquitin-proteasome pathway per se, we propose that Pin1 plays a critical role in controlling the *cis/trans* isomerization of phosphorylated substrate proteins targeted for ubiquitylation to establish the correct *cis/trans* conformation needed for SCF complex function (Figure 2).

At the molecular level, the mechanism by which Pin1 drives phosphorylation-dependent ubiquitylation remains unclear owing to a lack of structural evidence. Given a free energy barrier between *cis* and *trans* conformations, *cis/trans* interconversion would be rather slow, while it would be even slower after phosphorylation, which becomes a rate-limiting step for the phosphorylation-dependent ubiquitin-mediated proteasome degradation. Pin1 accelerates the rate of *cis/trans* conformational change by more than 100- to 1000-fold, thus providing the correct conformation and precise timing for further activation by the downstream E3 ligases and/or SCF complex activity, eventually leading to target protein degradation (Figure 2). Thus, we speculate that Pin1 might mediate phosphorylation-dependent ubiquitylation by regulating a specific *cis* or *trans* isomer of the phospho-substrates.

The ubiquitin E3 ligase complex might have a structural preference for its phosphorylated substrates selection. Recent structural study showed that the F-box protein of SCF^{cdc4} ubiquitin ligase complex binds to a *trans* phospho-Thr-Pro conformation of the substrate [29]. Furthermore, the Skp1-Skp2-Cks1-p27 complex structure demonstrates that Cks1 indeed interacts with a *trans* conformation of Thr187-Pro motif in p27 [84], further supporting our view that the E3 ligase or SCF complex has a structural preference for recognizing its substrates [85]. To some extent, Pin1 could convert the more stable *trans* prolyl conformation of substrates to become a *cis* conformation, which is unfavorable, in some cases, for the binding of E3 ligase or SCF complex, resulting in stabilization of the phospho-substrates [85]. As in tumour formation, Pin1 could prevent oncoproteins from

degradation by the phosphorylation-dependent ubiquitylation pathway via catalyzing its substrates from a *trans* conformation to a *cis* isomer (Figure 1). By contrast, Pin1 could catalyze a *cis* conformation to a *trans* conformation for cancer suppressors to induce their degradation during tumor cell proliferation. Through this mechanism with its unique enzymatic characteristic, Pin1 regulates multiple substrates involved in many different signaling pathways to synergistically drive certain cellular processes in one direction. Developing tools that are able to distinguish cellular *cis* and *trans* conformations of phosphorylated substrates *in vivo* would be extremely useful to elucidate how Pin1 regulates its substrate functions.

Pin1 regulates substrate dephosphorylation

The reversible phosphorylation of proteins on Ser/Thr-Pro residues is a key regulatory mechanism for the control of numerous cellular processes [86]. In addition to its role in regulating protein stability, Pin1 is also involved in the recycling of protein phosphorylation/dephosphorylation (Figure 1). Pro-directed PP2A activity is conformation specific and dephosphorylates only the *trans* phosphorylated Ser/Thr-Pro isomer [10]. Pin1 catalyzes the prolyl isomerization of specific phosphorylated Ser/Thr-Pro motifs in the mitotic phosphatase Cdc25C to facilitate PP2A-mediated Cdc25C dephosphorylation [10]. Indeed, Pin1 induces a conformational change in Cdc25 [87]. The phosphorylation-dependent conformational changes mediated by Pin1 represents a novel mechanism in regulating dephosphorylation of certain phosphorylated Ser/Thr-Pro in cellular signaling regulation.

Pin1-mediated phosphorylation/dephosphorylation recycling is also important in regulating tau in AD brains. Hyperphosphorylated tau abolishes its ability to bind microtubules and promote microtubule assembly, thus resulting in abnormal tau accumulation and neurodegeneration [88, 89]. Pin1 interacts with the Thr231-Pro motif of tau and thus facilitates PP2A-mediated dephosphorylation of hyperphosphorylated tau, thereby restoring the ability of phosphorylated tau to bind microtubules and promote microtubule assembly [10, 13, 67]. In addition to promoting PP2A-mediated dephosphorylation, Pin1 also plays an opposite role in downregulating PP2A activity. Neurofilament (NF) proteins are phosphorylated in the axonal compartment in normal neurons [90] and aberrantly hyperphosphorylated within the cell bodies of neurodegenerative disorders such as AD and PD [91]. In cortical neurons, Pin1 enhances the phosphorylation of Ser/Thr-Pro residues located within the tail domain of NF proteins by suppressing PP2A-mediated dephosphorylation [92], suggesting that the aberrant hyperphosphorylation of NFs in neurodegeneration can be attributed to the role of Pin1 in downregulating PP2A activity on its substrates.

Furthermore, Pin1 plays a pivotal role in regulating Ras/Raf-1 signaling by recycling hyperphosphorylated Raf-1 to its signal-competent stage, without affecting its protein stability [93]. The activation of Raf-1 kinase depends on the precise control of its phosphorylation and dephosphorylation state: whereas phosphorylation is required for Raf-1 kinase activity, MEK-dependent hyperphosphorylation induces a negative feedback mechanism and results in Raf-1 deactivation. Five out of six Raf-1 feedback phosphorylation sites are phosphorylated Ser-Pro motifs. Here, Pin1 catalyzes *cis/trans* isomerization of the hyperphosphorylated Raf-1 resulting in facilitating PP2A-mediated dephosphorylation of a *trans* form of Raf-1, thus recycling Raf-1 function in the signaling cycle [93]. More recently, a very interesting structural study revealed that Ssu72, a specific phosphatase for phospho-Ser⁵-Pro⁶ in the C-terminal (CTD) domain of RNA polymerase II, specifically dephosphorylates a *cis*-phospho-Ser⁵-Pro⁶ conformation of the CTD [9]. The *cis*-phospho-Ser⁵-Pro⁶ conformation comprises a minor population in solution, and the prolyl isomerase ESS1 (a yeast Pin1 homologue) enhances the dephosphorylation activity of

Ssu72 by at least 20-fold [9]. These results are consistent with the findings that Pin1 regulates RNA polymerase II protein phosphorylation and function in mitotic mammalian cells [94, 95]. Together, these results strongly support the idea that Pin1-mediated *cis/trans* isomerization could be a crucial regulatory mechanism in controlling the precise *cis*-proline or *trans*-proline isomer for downstream cellular events.

Molecular mechanisms of Pin1 regulation

Numerous studies have shown that Pin1 transcription, protein level, catalytic activity and function are tightly regulated by many different mechanisms under physiological conditions [3, 4]. Pin1 expression level is regulated by the transcription factor E2F, which specifically activates the *PIN1* promoter via E2F binding sites (Figure 3). Indeed, overexpression of E2F enhances *PIN1* promoter activity and mRNA level in breast cancer cells. In addition, Pin1 protein levels are enhanced by oncogenic Neu/Ras signaling through the activation of E2F [22]. Furthermore, a recent proteomics screen identified Pin1 as a target of mutant C/EBP α (C/EBP α -p30) in AML [40]. C/EBP α -p30 recruits E2F binding to the *PIN1* promoter, thus resulting in elevated Pin1 expression. Subsequently, Pin1 enhances the stability of c-Jun by preventing its ubiquitylation, thereby inhibiting granulocyte differentiation [40]. Cohort studies have also shown single-nucleotide polymorphisms (SNPs) that reduce Pin1 expression [96] and are associated with an increased risk for AD [97]. In contrast, a different SNP that prevents Pin1 transcriptional suppression by the brain-selected AP4 is associated with delayed onset of AD [98]. AP4 has been identified as a novel transcriptional repressor of Pin1 expression [98]. A functional polymorphism, rs2287839, in the Pin1 promoter prevents the ability of AP4 to bind and suppress Pin1 promoter activity, thus resulting in delayed onset of AD [98]. Taken together, these studies suggest that the complexity of the upstream regulatory mechanisms that control Pin1 might play a crucial role in controlling normal cellular function (Figure 3).

The phosphorylation status of Pin1 is highly regulated during cell cycle progression [99]. Plk-1, a key regulator of mitosis, binds and phosphorylates Ser65 in the catalytic domain of Pin1 [100]. This phosphorylation does not affect Pin1 enzymatic activity, but it does appear to regulate its stability by inhibiting its ubiquitylation and subsequent proteasome-mediated degradation. Conversely, depletion of Plk-1 by siRNA or ectopic expression of the dominant negative form of Plk-1 in HeLa cells results in enhanced Pin1 ubiquitylation and degradation [100]. Thus, Plk-1 is an upstream kinase of Pin1 that can stabilize Pin1 protein levels during mitosis. Additionally, PKA-mediated phosphorylation of Pin1 Ser16 can regulate the ability of Pin1 to interact with its substrates [3, 4]. More recently, death associated protein kinase1 (DAPK1), a well-characterized tumor suppressor [101] that can suppress c-Myc and E2F-induced oncogenic transformation [102], has been identified as a Pin1 inhibitory kinase [20]. DAPK1 phosphorylates Pin1 at Ser71, a catalytic site that inactivates Pin1 isomerase activity, thus inhibiting its nuclear localization and cellular function (Figure 3). These studies highlight that, in addition to the transcriptional regulation of Pin1 expression, Pin1 protein levels are also regulated through post-translational modifications. Given the vital role of Pin1 in various cellular functions and diseases, these findings provide great promise for the development of strategies to treat human disease.

Concluding remarks and future perspectives

Extensive research in the past 15 years has shown that Pin1-mediated *cis/trans* isomerization has profound effects on many cellular events and human diseases by controlling the fate of a wide variety of phosphorylated proteins. Moreover, Pin1 itself is regulated by different mechanisms at the transcriptional and post-translational levels. Importantly, it will be crucial to identify the upstream signal(s) that governs Pin1 function spatially and/or temporally.

Moving forward, a major challenge will be to determine if Pin1 can stabilize or destabilize a given substrate in response to different upstream stimuli.

Among its substrates, Pin1, in general, stabilizes oncoproteins and destabilizes or inactivates tumor suppressors, positioning Pin1 as a potential cancer therapeutic drug target. Pin1 is also involved in regulating the cross-talk of post-translational modifications, such as phosphorylation and ubiquitylation or sumoylation. Therefore, it will also be of interest to determine the role of Pin1 in regulating other post-translational modifications. Several recent studies provide new insights into the pivotal roles played by Pin1 in regulating stem cell renewal, viral replication and host pathogenesis (Box 2 and Box 3). A major challenge will be to develop tools to directly visualize Pin1-catalyzed protein conformational changes *in vivo* and to determine their roles in the development and treatment of human diseases. Moreover, Pin1 can bind non-canonical proline-rich motifs [103], indicating that it might also regulate non-phosphorylation-dependent pathways under certain circumstances. Indeed, the variety of recent findings related to Pin1 open new avenues for future studies aimed at understanding how Pin1 activity contributes to diverse cellular processes and disease conditions, thus highlighting its potential as a molecular target for novel drugs.

Box 2

Pin1 regulates protein stability in pluripotent stem cell proliferation

Embryonic stem cells (ESCs) can undergo unlimited self-renewal to retain their pluripotency, i.e. the ability to differentiate into a diverse range of specialized cell types. Characterization of pluripotent stem cell is often monitored by a range of transcription factors such as Oct4, Sox2, Klf4, Nanog and c-Myc. In 2007, Yamanaka's group showed that somatic cells could be reprogrammed to induced pluripotent stem cells (iPS) through the introduction of Oct4, Sox2, Klf4 and c-Myc [142, 143]. More recent studies have uncovered novel mechanisms, mediated by Pin1, that regulate specific intracellular phosphorylation signaling events in pluripotent cell proliferation [107, 108].

Nanog, an ESC-specific homeodomain protein is crucial in maintaining the self-renewal and genomic stability of ESCs [144]. In addition to the stringent transcriptional regulation, Nanog levels are also regulated by the post-translational modification mediated by Pin1 in mouse and human ESCs [107]. Nanog is phosphorylated at several Ser/Thr-Pro motifs; however, the protein kinases that mediate Pro-directed phosphorylation of Nanog are unknown. The phosphorylation of Nanog recruits Pin1 to bind Nanog, resulting in enhanced Nanog stability by inhibiting ubiquitylation, presumably due to induced conformational changes catalyzed by Pin1 [107]. Inhibition of Pin1 activity or disruption of the Pin1–Nanog interaction suppresses ESC self-renewal and teratoma formation in immunodeficient mice [107].

Pin1 is upregulated upon cellular reprogramming; this elevated expression of Pin1, in addition to the expression of defined reprogramming factors, can enhance the frequency of iPS cell generation [108]. Conversely, inhibition of Pin1 reduces colony formation and stimulates aberrant differentiation of human iPS cells and murine ESCs. Oct4 was identified as a putative Pin1 substrate in human iPS cells; Pin1 interacts with Oct4 through a phosphorylated Ser12-Pro motif, thereby enhancing its stability and transcriptional activity [108]. Taken together, Pin1 appears to play a role in modulating the transcription factor network that regulates pluripotent stem cell proliferation and governs the self-renewal and maintenance of pluripotency in ESCs and iPS cells.

Box 3

Pin1 regulates protein stability in viral replication and infection

Recent reports indicate that the stability of several viral proteins is regulated by the PPIase activity of Pin1. These studies unravel a novel function of Pin1 in the pathogenesis of hepatocarcinogenesis, human T-cell leukemia virus type-1 (HTLV-1)-related diseases and viral infection [83, 109–111]. Hepatitis B virus (HBV), a known etiologic agent in hepatocellular carcinoma, encodes hepatitis B virus X protein (HBx), an oncogenic Pin1 substrate [111]. Pin1, which is upregulated by HBV infection, binds HBx at the phosphorylated Ser41-Pro motif and increases its stability and pro-tumorigenic activity [111]. The mitogen-activated protein kinase (MAPK) family was hypothesized to play a role in the phosphorylation at the Ser41-Pro site of HBx given that the HBx–Pin1 interaction is abolished by a MEK inhibitor [111]. Stabilized HBx might, in turn, enhance transactivation of genes involved in cell proliferation.

Similarly, Pin1 can enhance HTLV-1-induced T cell leukemia [83, 109]. The oncogenic HTLV-1 Tax protein is essential for viral replication and the malignant transformation properties of HTLV-1 in adult T-cell leukemia (ATL) [109]. Pin1 is highly expressed in ATL cells expressing Tax, and increased Pin1 expression enhances Tax protein expression. Pin1 functionally interacts with the HTLV-1 Tax oncoprotein at the phosphorylated Ser160-Pro site, thus stabilizing Tax by suppressing its polyubiquitylation and subsequent lysosomal degradation [83, 109]. The Pin1–Tax interaction plays a supporting role in Tax-mediated cell transformation and functionally enhances Tax-induced NF- κ B activation [83, 109]. Finally, during HIV infection, Pin1 plays a central role in the regulating viral DNA integration in activated T lymphocytes [110]. HIV integrase (IN) is a Pin1 substrate; the Pin1 – HIV IN interaction relies upon JNK-mediated phosphorylation of the HIV IN Ser57-Pro motif. Pin1-dependent increases in HIV IN steady-state levels enhance HIV IN activity, thereby facilitating HIV-1 proviral integration into the host cell genome [110].

These findings revealed the previously unknown role of Pin1 in regulating viral replication by modulating viral protein stability and function. Therefore, it will be of interest to uncover additional novel Pin1 targets from viral proteins of different species to elucidate the underlying mechanisms of molecular etiology of virus–host cell interactions.

Acknowledgments

We are sorry for not able to include all references on Pin1 due to space limitations. We are grateful to the members of Liou, Zhou and Lu laboratories for stimulating discussions and figure preparation, particularly to L. Tan, J.Y. Goh and F. Ye. K.P. Lu is a Senior Investigator of the American Asthma foundation. This work was supported in part by grants (09/1/21/19/604, SSCC-09-020) from the BMRC and (MOE2009-T2-2-111) from MOE, Singapore to Y.C.L., NIH R01CA122434 to X.Z.Z., and NIH R01GM058556 and AG017870 and Alliance for Lupus Research grant to K.P.L.

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Figure 1. Pin1 is a molecular switch to determine the fate of phosphoproteins

Pin1 regulates phosphorylated proteins through several mechanisms. (a) Recycle phosphoproteins; by controlling the conformation of specific phosphorylated Ser/Thr-Pro motifs, Pin1 regulates the function of its phospho-substrates by modulating their phosphorylation/dephosphorylation state. (b) Enhance degradation; Pin1 would boost *cis/trans* isomerization rate to grant the right conformation for subsequent ubiquitylation of phospho-proteins by E3 ligase and activate proteasome mediated protein degradation. (c) Prevent degradation; Pin1 could render the protein unfavorable for ubiquitin ligase binding or inhibit ubiquitin ligase access to the phosphorylated sites to increase the half-life of the substrate protein. (d) Regulate other post-translational modifications (PTMs); Pin1 regulates the cross-talk between several post-translational modification mechanisms such as phosphorylation and ubiquitylation (Ub); phosphorylation and sumoylation (sumo) and others such as acetylation (Ac).

Figure 2. Pin1-catalyzed prolyl *cis/trans* isomerization could be a crucial step in controlling the phosphorylation-dependent ubiquitylation pathway

Pin1 plays a critical role in regulating phosphorylation-dependent ubiquitylation pathway by catalyzing the intrinsically slow prolyl *cis/trans* isomerization. The catalytic processes of phosphorylation and ubiquitylation occur very rapidly under normal condition. However, the *cis/trans* conformation conversion of phosphorylated protein which is crucial for SCF complex or E3 ligase recognition occurs slowly. In the absence of Pin1, the *trans* to *cis* conformation conversion occurs very rarely due to difficulty in overcoming a high predicted energy barrier ($\Delta G_{tc}=30$ kcal/mol) of this conformational change. In addition, using NMR, the rate of the *cis* to *trans* conversion is shown to be slow on the order of $<0.002/\text{sec}$ at 25°C [3]. However, in the presence of Pin1, Pin1 enhances the *cis/trans* conformational changes by reducing the free energy barrier, resulting in a markedly increased conversion rate up to 100- to 1000-fold. Therefore, the prolyl *cis/trans* isomerization mediated by Pin1 could be a critical step in controlling the phosphorylation-dependent ubiquitylation pathway.

Figure 3. Molecular mechanisms of Pin1 regulation

The expression of Pin1 is regulated by both transcriptional regulation and post-translational modification. In transcriptional regulation, Pin1 is regulated by transcription factor E2F, which activates *PIN1* promoter specifically through the E2F binding sites. Moreover, different SNPs can activate or suppress Pin1 protein expression. The transcriptional repressor AP4 binds to a repressor SNP (rSNP) and thus suppresses Pin1 promoter activity. However, Pin1 could also regulate through activator SNPs (aSNPs). Through the post-translational modification, DAPK1 phosphorylates Pin1 at Ser71 residue in the PPIase catalytic site and inactivates the Pin1 catalytic activity presumably because the phosphate might form a hydrogen bond with the critical residue Arg69, and thus inhibits Pin1 nuclear localization and cellular function. Similarly, Pin1 is phosphorylated at Ser16 in the WW domain possibly by PKA and this phosphorylation inhibits the Pin1 WW domain to bind substrates presumably because the phosphate might form a hydrogen bond with the critical residue Arg17.

Table 1

List of selected Pin1 substrates

Pin1 prevents protein from degradation					
Protein	Pin1 function	Pin1 binding motif	Kinase/phosphatase	Pathways/diseases/mechanisms	Refs.
Cyclin D1	Stabilization; localization and transcription	Thr286	GSK-3 β	Cell proliferation, cancers	[11]
β -Catenin	Stabilization, localization and transactivation	Ser246	Could stabilize β -catenin by inhibiting GSK-3 β dependent degradation	Cell proliferation, cancers	[14]
p65/NF- κ B	Nuclear translocation, Stabilization	Thr254		Cytokines, hepatocyte NF- κ B activation	[15]
p53	Stabilization and increase p53 promoter binding activity; enhances formation of high molecular weight complexes (HMWC) and stabilizes p53	Ser33, Ser315, Thr181, Pro82	Chk2	Genotoxic stress, DNA damage, trophoblast invasiveness	[16, 18, 19, 104, 105]
c-Jun	Transcriptional activity	Ser63/Ser73	JNK	Breast cancer, AML	[17, 40]
Mcl-1 (Myeloid cell leukemia sequence-1)	Inhibits Mcl-1 ubiquitylation, stabilizes Mcl-1	Thr163; Thr92	JNK3 induces Mcl-1 degradation; Erk pathway	Oligodendrocyte apoptosis, Mcl-1 mediated chemoresistance, breast cancer	[43, 106]
Spt23	Ess1 stabilizes Spt23	Ser654		Unsaturated fatty-acid synthesis	[105]
p73	Enhances stabilization, and increases transcriptional activity	Ser412, Thr442 and Thr482	c-Abl and p300	Genotoxic stress	[82]
Synphilin-1	Facilitates Lewy body formation and stabilizes alpha-synuclein	Ser211 and Ser215	casein kinase II	Parkinson disease	[72]
p27	Protects p27 from degradation	Thr187	Cdk2	Cell cycle, cancers	[85]
Nanog	Enhances Nanog stability	Ser52, Ser65		Stem cells pluripotency, cell renewal	[107]
Oct4	Enhances Oct4 stability and transcriptional activity	Ser12-Pro		Stem cells pluripotency, cell renewal	[108]
HTLV (human T-cell leukemia virus) Tax protein	Increases Tax protein expression and inhibits Tax protein degradation	Ser160		Pathogenesis of HTLV-1 related diseases	[109]
Viral integrase	Stabilizes phospho- HIV-1 integrase	Ser57	JNK	HIV-1 cDNA integration and infection	[110]
HBx (Hepatitis B virus encoded protein X)	Increases stability of HBx	Ser41	Hepatitis B virus- infected hepatocytes	Hepatocarcinogenesis	[111]
c-Fos	Enhances stability and transcriptional activation	C-terminal	ERK		[112]
Sil	No impact on Sil spindle checkpoint			Unknown	[113]
p54nrb		Thr412, Thr430 and Thr452	Cdk1		[114]

Pin1 prevents protein from degradation					
Protein	Pin1 function	Pin1 binding motif	Kinase/phosphatase	Pathways/diseases/mechanisms	Refs.
AUF1 (AU rich element-binding protein)	Regulates GM-CSF mRNA	Ser83			[115]
BIM _{EL}	Stabilizes BIM _{EL} and induces apoptosis	Ser65	JIP3, MKK7 and JNK	Neuronal apoptosis	[116]
ORC1 (origin recognition complex, subunit 1)	Prevents degradation of ORC1 by inactivating mitotic APC complex		APC; Topo II	Mitosis, chromosome segregation and for reprogramming replicons	[117]
Bcl-2	Induces changes in the bioactivity of Bcl-2; Prevents dephosphorylation of Bcl-2		Possibly mediated by cdc2		[118, 119]
ErbB2	Stabilizes ErbB2	Ubiquitinated ErbB2	ErbB2 pathway; ubiquitin mediated degradation	Her2-positive breast cancer	[120]
PPAR γ	Prevents the polyubiquitylation of PPAR γ through ubiquitin-proteasome pathway	Ser84	Ras mediated kinase	Macrophages mediated atherosclerosis	[121]
Cep55	Increases Cep55 stability	Ser425, Ser428	Cdk1, Plk1	Mitosis and cytokinesis	[122]
Akt	Regulates Akt protein stability	Thr92, Thr450		Oncogenesis	[123]
AGL24	Enhances stability	Thr202		Control flowering in Arabidopsis	[124]
SOC1	Enhances stability	Ser49 and Ser195		Control flowering in Arabidopsis	[124]

Pin1 enhances degradation of proteins					
Protein name	Pin1 function	Pin1 binding motif	kinase/phosphatase	Pathways/diseases/Mechanism	Refs.
c-Myc	Enhances c-Myc degradation	Thr58		Tumorigenesis	[46]
Cyclin E	Depletion of Pin1 upregulates cellular level of cyclin E	Ser384	Cyclin E-Cdk2 complex	Cell cycle, genomic instability and tumorigenesis	[48]
RAR α	Induces RAR α degradation	Ser77		Retinoic acid receptor	[50, 51]
PML (promyelocytic leukemia protein)	Enhances PML degradation	C-terminal of PML (Ser403, Ser505, Ser518 and Ser527)		Breast cancer, hydrogen peroxide-induced death, cell proliferation	[44]
SRC-3/AIB1	Enhances SRC-3 degradation	N/A	Steroid receptor		[53]
Smad2/Smad3	Reduces Smad2/3 protein levels	Thr179, Ser204, Ser208, Ser213 (Smad3 linker domain)	Smurf2 with Smads and enhanced Smad ubiquitylation	TGF- β signaling	[58]
GRK2 (G protein-coupled receptor kinase 2)	Promotes GRK2 degradation	Ser670	CDK2-cyclinA	Cell cycle, p53 response and the induction of apoptosis	[63]
Tau	Pin1 knockdown or knockout increased wild-type tau protein stability <i>in vitro</i>	Thr231	Cdk5, p38, MAP kinases	Tauopathy	[70]

Pin1 enhances degradation of proteins					
Protein name	Pin1 function	Pin1 binding motif	kinase/phosphatase	Pathways/diseases/Mechanism	Refs.
Che-1	Pin1 is requested for Che-1/HDM2 interaction	Thr144		p53 transcription, DNA damage apoptotic pathway	[81]
TRF-1	Promotes TRF1 degradation	Thr149	Cdk	Cancer, ageing	[79]
Btk (Bruton tyrosine kinase)	Mediates Btk degradation	Ser21 and Ser115		Tyrosine kinase	[125]
IRF3	Promotes its degradation via the ubiquitin-proteasome pathway.	Ser339		Host antiviral responses during virus infection	[126]
SMRT (silencing mediator for retinoic acid and thyroid hormone receptor)	Destabilizes SMRT	Ser1241, Thr1445, Ser1469	Her2/Neu/ErbB2 receptor; Cdk2	Human cancer	[127]
Pim-1 protein kinase	Destabilizes Pim-1	N/A	PP2A	Elevated in lymphomas leukemias and prostate cancer through c-Myc pathway	[128]
FOXO	Interconnecting FOXO phosphorylation and monoubiquitylation in response to cellular stress to regulate p27			Oxidative stress	[129]
SF-1	Promotes SF-1 ubiquitylation and degradation	Ser203	CDK7	Gonadotropin beta-subunit gene transcription	[130]
SUL/T4A1 (sulfotransferase 4A1)	Destabilizes SUL/T4A1	Thr8, Thr11	ERK1 and PP2A	Metabolism of endogenous and exogenous compounds	
MEF2C	Decreases MEF2C stability	Ser98/Ser110		Muscle terminal differentiation	
Daxx	Enhances Daxx degradation	Ser178-Pro	ASK1/JNK kinases	oxidative stress-induced cellular apoptotic response	

Pin1 regulates phosphorylation/dephosphorylation of proteins					
Protein	Pin1 function	Pin1 binding motif	kinase/phosphatase	Pathways/diseases/mec hanism	Refs.
Tau	Enhances dephosphorylation at Thr231	Thr231		Neuronal differentiation, induced stress, Alzheimer disease	[12, 13, 135]
Raf-1	Enhances dephosphorylation by PP2A	N/A	Ras/MAP kinase		[93]
I-2 (type-1 protein phosphatase Inhibitor-2)	Cycling phosphorylation in mitotic	Thr72	CDK1-cyclin B	Entry and exit in mitotic	[136]
TGF-β1 mRNA	accumulation and translation of TGF-β1 mRNA in Eosinophils			Chronic asthma	[137]
KSRP (K-homology splicing regulator protein)	Regulates parathyroid hormone (PTH) mRNA stability	Ser181			[138]