REVIEW

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Prolyl *cis-trans* isomerization as a molecular timer

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Proline is unique in the realm of amino acids in its ability to adopt completely distinct *cis* and *trans* conformations, which allows it to act as a backbone switch that is controlled by prolyl *cis-trans* isomerization. This intrinsically slow interconversion can be catalyzed by the evolutionarily conserved group of peptidyl prolyl *cis-trans* isomerase enzymes. These enzymes include cyclophilins and FK506-binding proteins, which are well known for their isomerization-independent role as cellular targets for immunosuppressive drugs. The significance of enzyme-catalyzed prolyl *cis-trans* isomerization as an important regulatory mechanism in human physiology and pathology was not recognized until the discovery of the phosphorylation-specific prolyl isomerase Pin1. Recent studies indicate that both phosphorylation-dependent and phosphorylation-independent prolyl *cis-trans* isomerization can act as a novel molecular timer to help control the amplitude and duration of a cellular process, and prolyl *cis-trans* isomerization might be a new target for therapeutic interventions.

Biological processes in the cell are extremely dynamic and complex events that are superbly choreographed both spatially and temporally. These events are often controlled by networks of tightly regulated intermolecular interactions and enzymatic reactions that involve a specific subset of proteins within a cell. The location and timing of these events, which can also be tightly regulated, often play key roles in determining the output of a given process. The proper modulation of protein function is central to this orchestration. A number of regulatory mechanisms have been well established, including post-translational chemical modifications of selected amino acid side chains, allosteric regulation, and regulated protein degradation. Recently, the contribution of protein conformational changes—and in particular the intrinsic conformational switch afforded to peptides by prolyl *cis-trans* isomerization—to the regulation of cellular processes has begun to be appreciated[1–7].

Proline residues in proteins can exist in completely distinct *cis* and *trans* peptide bond conformations and thereby provide an intrinsic backbone switch that is controlled by prolyl *cis-trans* isomerization (**Fig. 1**). This intrinsically rather slow conversion can be catalyzed by ubiquitous enzymes called peptidyl prolyl *cis-trans* isomerases (PPIases) or rotamases, which can be divided into four structurally unrelated families: cyclophilins (Cyps), FK506-binding proteins (FKBPs), parvulins and the recently identified protein Ser/Thr phosphatase 2A (PP2A) activator PTPA. Cyclophilins and FKBPs have been the subject of intense research because they are cellular targets for the clinically used immunosuppressive drugs cyclosporin A (CsA) and FK506, respectively, and FKBPs are also targets for the immunosuppressant and anticancer agent rapamycin[8–11]. However, it turns out that the action of these drugs does not involve the inhibition of PPIase activity *per se*, but instead it involves the promotion of the formation of a ternary complex with calcineurin or target of rapamycin (TOR), which results in the inhibition of calcineurin phosphatase or TOR kinase activity, respectively[12–16]. Moreover, all eight Cyps and four FKBPs are dispensable individually and together for viability in budding yeast[17]. However, these proteins are known to be important in the function of particular proteins. For instance, the Cyp NinaA is required for the synthesis and localization of some *Drosophila melanogaster* rhodopsins[18]. In addition, CypA interacts specifically with the human immunodeficiency virus (HIV) capsid protein[19], and Cyp40 is part of the Hsp90–Hsc70 complex that binds steroid receptors[20]. Likewise, FKBP12 is a subunit of the ryanodine receptor and the IP3 receptor[21] and also inhibits type 1 TGF-β receptors[22]. Therefore, PPIases were thought to perform nonessential cellular roles such as facilitating protein folding or participating in protein complexes as chaperones or integral subunits, and doubt was cast on their value as therapeutic targets.

The discovery of the PPIase Pin1 has cast a new light on the cellular importance of prolyl *cis-trans* isomerization. Human Pin1 was originally identified by its ability to interact with *Aspergillus nidulans* NIMA, a mitotic kinase phosphorylated on multiple Ser/Thr-Pro motifs during mitosis, and its ability to suppress the induction by NIMA of mitotic catastrophe in budding yeast[23]. Unlike all other known PPIases, Pin1 binds to and isomerizes specific phosphorylated Ser/Thr-Pro (pSer/Thr-Pro) motifs in certain proteins (**Fig. 1b**), which led to the hypothesis of a new signaling mechanism whereby Pin1 catalytically regulates the conformation of its substrates after phosphorylation to control protein function[1,2,24,25]. Recent studies have established that Pin1-catalyzed prolyl isomerization can have a profound impact on many key proteins in diverse cellular processes, including cell growth regulation, genotoxic and other stress responses, immune response, germ cell development,

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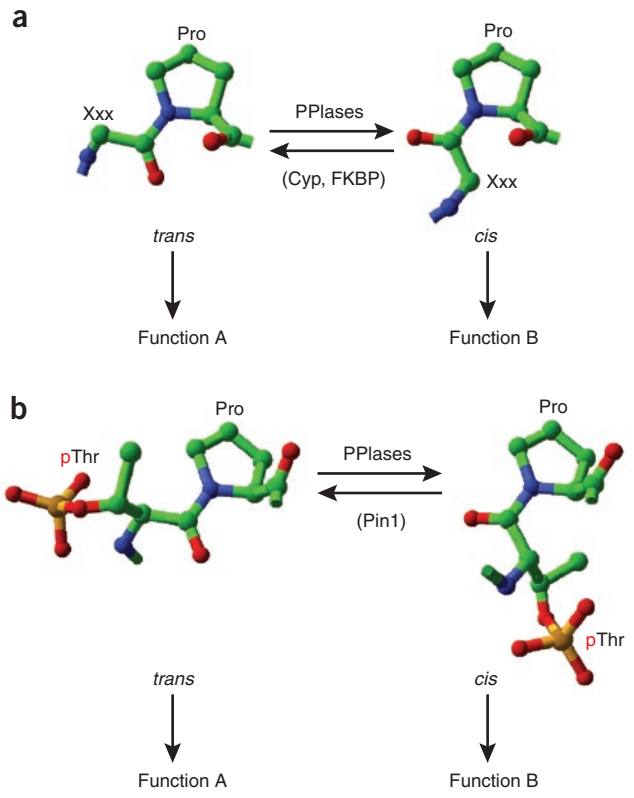

Figure 1 Prolyl *cis-trans* isomerization as a molecular switch. The local environment of proline within a protein can influence the relative free energies of the *cis* and *trans* isomeric states, leading to wide variations in the ratio of *cis*:*trans* populations in different proteins. Although most structures require proline 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 proline residues[4]. Owing to the relatively large energy barrier, uncatalyzed isomerization is a rather slow process, but it can be greatly accelerated by PPIases. (**a**,**b**) Based on the substrate specificity, PPIases can be divided into phosphorylation-independent and phosphorylation-dependent enzymes. The former group includes Cyps, FKBPs, PTPA and many parvulins that catalyze isomerization of Xxx-Pro motifs, where Xxx indicates any amino acid except pSer or pThr (**a**). Pin1 and Pin1-type enzymes are the only known phosphorylation-dependent PPIases that isomerize on the pSer/Thr-Pro motifs (**b**). The requirement of substrate phosphorylation for Pin1-type enzyme activity imparts an additional level of regulation that couples this isomerization switch to the tightly regulated activities of kinases and phosphatases[47–49]. Recent studies indicate that the *cis* and *trans* isomers of many proteins have distinct functions, and their conversions by PPIases can function as a new general class of protein regulatory switches.

and neuronal differentiation and survival[5,7,26–29]. Pin1 has emerged as a novel molecular timer that synergistically controls the amplitude and duration of a cellular response or process under a given condition. Importantly, Pin1 is tightly regulated at multiple levels, and its deregulation has a critical role in a growing number of pathological conditions, including cancer, Alzheimer's disease, aging, asthma and microbial infection[5,7,26–29]. Moreover, recent studies have uncovered that phosphorylation-independent prolyl isomerization can also function as a molecular timer in a number of biological and pathological processes, including cell signaling, ion channel gating, gene expression and infection[3,4,6,30–33].

This review will focus on the molecular and structural basis for prolyl *cis-trans* isomerization as an intrinsic conformational switch. We will also discuss the regulatory role of this general mechanism as a molecular timer in human physiology and pathology.

Prolyl *cis-trans* isomerization as a fundamental switch

The peptide bond linking adjacent amino acid residues in a protein backbone can adopt either the *trans* or *cis* conformation (**Box 1** and **Fig. 2**). For all amino acid residues except proline, the *trans* conformation is far more energetically favorable than the *cis*. For proline, with a peptidyl-prolyl imide bond, the free energy difference between the *cis* and *trans* isomers is much smaller. In fact, the *cis* conformation occurs with a frequency of 5–6% in protein structures[34,35]. Moreover, a large majority of the observed *cis* peptide bonds occur in surface-accessible bend, coil or turn conformations[36,37]. Thus, Xxx-Pro motifs in proteins have the potential to adopt two distinct conformations, *cis* and *trans*, and are typically solvent-exposed on the surface of proteins.

The structural difference between these two isoforms constitutes a fundamental molecular switch that can, for example, toggle between two functional states of the protein in which it resides, or distinguish between two separate sets of intermolecular binding partners (**Fig. 1**).

The high-energy barrier associated with the $\omega = 90°$ *syn* transition state imparts a very slow timescale (typically many minutes) to the intrinsic isomerization process (**Box 1**). This slow intrinsic exchange rate effectively isolates the *cis* and *trans* isomer pools from each other relative to the fast timescale of biochemical pathways in the cell, and if one population is selectively depleted, the equilibrium cannot rapidly be restored without the assistance of a catalyst.

Catalysis of prolyl *cis-trans* isomerization by PPIases

So far, four structurally unrelated families of PPIase enzymes have been identified: cyclophilins, FKPBs, parvulins and PTPA (**Fig. 3**). The catalytic domains of the cyclophilin, FKPB and parvulin families all have a central β-sheet and function as monomers[24,38,39]. Surprisingly, the catalytic domain of PTPA is an all-α-helix fold with the active site located at the interface of a substrate-induced dimer[40]. An important first question to address regarding the mechanism of PPIase action is which end of the substrate is rotated relative to the enzyme during the isomerization reaction. This question was apparently answered for CypA, as comparison of X-ray structures of the enzyme complexed with *cis* and *trans* isomers of HIV-1 capsid mutants clearly showed an anchored proline position and rotation of the sequence N-terminal to the isomerized N-C′ bond (for example, see **Fig. 1**)[41]. However, subsequent solution NMR dynamics studies of CypA in action on a four-residue peptide substrate were interpreted in support of a C-terminal rotation mechanism[42]. A recent molecular dynamics study of CypA and the same peptide supports the N-terminal rotation mechanism and asserts that this mechanism is consistent with the previously reported NMR data[43]. Interestingly, a recent NMR dynamics study of the catalytic domain of Pin1 suggests an N-terminal rotation mechanism as well[44]. This question of "which end wags" has not yet been addressed for PTPA and FKBP.

PPIases function as accelerating agents, speeding up the *cis*-to-*trans* and *trans*-to-*cis* conversion rates by several orders of magnitude[7,45]. The fundamental principle of enzyme catalysis is that an enzyme accelerates a specific reaction by lowering the activation barrier for the reaction and/or raising the energy of the bound substrate[46]. For the case of PPIases, the *cis* and *trans* isomers can each act as either substrate or product, and the transition state is approximated by the high-energy, "twisted" *syn*-90 state. The environment of the active site of a PPIase must, then, provide a mechanism by which the free energy of the enzyme–*syn*-90 complex is brought closer to the free energies of the enzyme–*cis* and enzyme–*trans* ground state complexes, which is equivalent to lowering the torsion bar-

rier for rotation about the N-C′ peptide bond. Despite extensive studies, the precise structural and chemical mechanisms of catalysis remain unclear, with several possibilities proposed (**Box 2**)[45].

Regardless of how it is accomplished, PPIase-catalyzed isomerization provides an "open conduit" on the millisecond timescale between *cis* and *trans* isomer populations in the cellular environment[7]. In the absence of PPIase, if either the *cis* or *trans* isomer were depleted (for example, via isomer-specific interactions), restoration of equilibrium *cis* and *trans* isomers would require many minutes (**Box 1**). Through its acceleration of isomerization, specific PPIase activity maintains equilibrium populations of targeted *cis* and *trans* substrate conformations on the millisecond timescale, which is more relevant for the regulation of dynamic biological processes. The precise timing of *cis-trans* conformational exchange depends explicitly on the enzyme-catalyzed activation barrier (**Box 1**), and therefore on the active site environment and the substrate. Therefore, *cis-trans* isomerization is a molecular switch that can be used in an enzyme-regulated manner to control the timing of biological events such as isomer-specific protein-protein interactions.

Unlike other known PPIases, Pin1 uses substrate Ser/Thr phosphorylation as an additional level of regulation (**Fig. 1b**)[1,5,23,24,26]. Only when a substrate is phosphorylated is the *cis-trans* isomerization accelerated. Isomerization of pSer/Thr-Pro motifs is especially significant because proline-directed kinases and phosphatases are conformation-specific, acting only on the *trans* conformation[47–49]. Moreover, phosphorylation further dramatically slows down the intrinsic isomerization rate of Ser/Thr-Pro bonds, and renders the peptide bonds resistant to the catalytic action of other known PPIases[1]. Another unique aspect of Pin1 is the separation of its binding and catalysis functions into distinct domains: WW and PPIase, respectively (**Fig. 3**)[1,24,25]. Evidence acquired so far supports the original view that the WW domain is required for Pin1 function by targeting the catalytic PPIase domain to its substrates[7,25,26,50]. Though simple mass action can explain this requirement by covalent attachment of the WW domain, recent NMR studies indicate that there may be dynamic interactions between these two domains, at least *in vitro* (**Fig. 3**)[7,51–54]. The emerging picture shows Pin1 as being rich in functionally relevant motions, with contributions of dynamics to substrate selection and transdomain communication and to the physical process of isomerization at the active site.

Prolyl *cis-trans* isomerization as a molecular timer

Cell cycle. Progression through different phases of the cell cycle is driven by the timely activation and inactivation of different proline-directed cyclin-dependent kinases (Cdks)[55]. However, given that Cdks often phosphorylate many substrates (for example, several hundred proteins are phosphorylated by Cdc2 during the G2/M transition[56]), it remains largely unknown how these phosphorylated proteins are coordinated to induce highly choreographed cell cycle events. Recent results suggest that Pin1-catalyzed postphosphorylation regulation might be an important mechanism in coordinating mitotic progression and modulating the transition between G0/G1 and S phase.

Consistent with its original identification[23], Pin1 affects mitotic progression in yeast and cancer cells[23,50] and affects the DNA replication–mediated mitotic checkpoint and G2/M transition in *Xenopus laevis* cell cycle extracts[57,58]. Furthermore, Pin1 targets to a large number of mitosis-specific phosphoproteins, which are also recognized by the phosphospecific mitosis marker MPM-2 monoclonal antibody[1,59,60]. For

Box 1 The activation energy and intrinsically slow rate of peptidyl prolyl *cis-trans* isomerization

The fundamental repeating unit of a protein backbone is the six-atom peptide plane, which contains three atoms from each of two adjacent amino acid residues. This planar unit can be classically viewed as 40% double-bond character of the N-C′ bond due to delocalization of the nitrogen lone electron pair. Rotation about the central bond of the peptide plane is highly restricted. Although the conformation of a protein backbone is defined by three torsion angles per amino acid residue (ϕ, ψ and ω), for all amino acid residues except proline the *trans* conformation (ω = 180°) is far more energetically favorable than the *cis* (ω = 0°), and protein structure is typically expressed in terms of the ϕ and ψ torsion angles with ω = 180°. However, because of the unique backbone-linked five-membered ring of proline and the corresponding imide peptide bond, the *cis* and *trans* isomers are closer in free energy for the Xxx-Pro peptide bond (where Xxx is any residue), and the *cis* conformation appears with a frequency of 5–6% in protein structures[34,35]. Long before high-resolution protein structures were available, Linus Pauling proposed a simple approximate relationship for the strain energy associated with rotation about the C-N bond of a peptide plane[127]:

$E = 30 \sin^2 \delta$ (kcal mol^{-1})

where δ is the dihedral angle measuring deviations from the *trans* conformation. A recent analysis of 3,938 high-resolution protein structures demonstrates the remarkable accuracy of this early approximation[128]. In agreement with this prediction, small deviations from planar geometry (±5°) are frequently observed in actual protein structures, while deviations of 15–20° or more are not. Based on Pauling's prediction, the 90° (*syn*) transition state has an energy that is 30 kcal mol^{-1} higher than that of the 180° (*trans*) conformation, which indicates that conversion from the *trans* to *cis* state would be expected to occur only rarely. Indeed, NMR measurements for *cis*-to-*trans* conversion in peptides show activation barriers $\Delta G^{\dagger}_{ct}$ ranging from 14–24 kcal mol^{-1}, while rate measurements by NMR and by proteolytic or protease-free assays confirm slow *cis*-to-*trans* conversion rates on the order of 0.002 s^{-1} at 25 °C (refs. 1,49,129). The lower free energy of the *trans* state relative to the *cis* state would correspond to a higher activation barrier for the *trans*-to-*cis* conversion, thereby bringing the activation barrier $\Delta G^{\dagger}_{tc}$ closer to Pauling's predicted 30 kcal mol^{-1}.

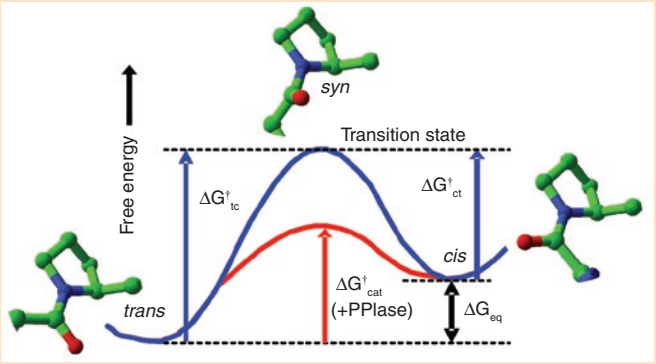

Figure 2 Energy diagram for prolyl *cis-trans* isomerization. Conformational exchange proceeds through the twisted 90-*syn* high-energy transition state in the intrinsic (blue curve) or PPIase-catalyzed (red curve) reactions.

Box 2 How might PPIases overcome the barrier to peptide bond rotation?

The precise mechanisms of action at work in PPIases are still not entirely understood. Several proposed mechanisms for the overall reduction of the N-C′ torsion barrier by 6–8 kcal mol^{-1} have been considered[130,131] and significant ones are briefly described here.

Substrate desolvation. The substrate desolvation (or hydrophobic) mechanism is based on the finding that interactions between the carbonyl oxygen of the peptide plane and polar groups such as water molecules increase the π-bond overlap population, thereby increasing the *cis*-to-*trans* torsion barrier over that found in apolar environments[132,133]. Reduction of the *cis*-to-*trans* torsion barrier is thus expected for transfer of the substrate from an aqueous environment into an enzyme active site in which the carbonyl oxygen does not interact with polar groups. This is an example of ground state destabilization, as resonance of the peptide bond in each of the *cis* and *trans* states involves charged species that are disfavored in hydrophobic surroundings. Substrate desolvation is estimated to contribute between 1.3 and 1.8 kcal mol^{-1} toward lowering the N-C′ torsion barrier in proline-containing peptides, based on studies of peptide isomerization in apolar solvents[132] and in micelles and membranes[134]. This mechanism is currently thought to make a relatively small contribution to the overall reduction of the N-C′ torsion barrier by PPIase enzymes, and it was invoked to rationalize the relatively weak isomerase activity of monoclonal antibodies raised against haptens meant to mimic the twisted transition state[135,136].

Substrate autocatalysis. In this mechanism, intramolecular interactions within the substrate that stabilize the transition state make a small contribution to catalysis. Such interaction might arise from a hydrogen bond between the imide nitrogen lone pair and the NH of the amino acid following proline[137].

Preferential transition-state binding. For this "conformational twist" mechanism, the geometry and chemical features of the enzyme active site provide specificity for favorable interactions with the twisted *syn*-90 conformation, whereas less favorable interactions are possible with either the *cis* or *trans* isomers[131,137]. Possible scenarios include electrophilic stabilization of the nitrogen lone pair, and the involvement of a water molecule to stabilize the transition state[45].

Nucleophilic catalysis. This mechanism involves formation of a covalent tetrahedral intermediate in which a nucleophilic atom of the enzyme forms a covalent bond with C′ of the substrate[24]. The resulting tetrahedral geometry at C′ abolishes the previous resonance stabilization of the peptide bond, and the torsion barrier for N-C′ rotation is greatly reduced. Required in this mechanism is nucleophilic attack on the prolyl carbonyl carbon by an activated enzyme group, such as a sulfhydryl group of a cysteine side chain.

example, Pin1 catalyzes conformational changes in the mitosis-inducing phosphatase Cdc25C to regulate its catalytic activity directly[58,59] or indirectly via promoting its dephosphorylation[49]. Pin1 also inactivates the mitosis-inhibitory kinase Wee1 at M phase by impairing the function of the Wee-box[61]. In addition, Pin1 acts on the anaphase-promoting complex early mitotic inhibitor 1 (Emi1) to prevent its degradation during G2 phase, which is required to induce M-phase entry by stimulating the accumulation of cyclin B (ref. 62). Finally, Pin1 interacts with mitotic phosphoproteins on chromatin, especially TopoIIα, and regulates their phosphorylation and chromosome condensation[63]. Given that Pin1 substrates affect different mitotic events or are distributed at various subcellular structures during mitosis, and that Pin1 depletion leads to mitotic catastrophe in cells[23,50], these results suggest that Pin1 might help coordinate mitotic signaling events after substrate phosphorylation and provide a means for temporally and spatially orchestrating the activity of mitotic proteins into an abrupt wave of signaling that proceeds in a synchronous manner.

Pin1 also has an important role at the transition between G0/G1 and S phase. For example, Pin1 regulates transcription of cyclin D1 in response to multiple upstream growth signal pathways[64–66] and also directly acts on cyclin D1 to increase its protein stability and nuclear localization[67]. In addition, Pin1 increases protein turnover of c-Myc and cyclin E (refs. 68,69). Importantly, Pin1 knockout mouse embryonic fibroblasts (MEFs) show defective cell cycle re-entry[70] and G1/S transition[69]. Moreover, Pin1 also coordinates centrosome duplication and DNA synthesis[71]. Pin1 specifically localizes to centrosomes during S phase, and its ablation delays centrosome duplication during S phase synthesis[71]. In contrast, Pin1 overexpression induces centrosome amplification, chro-

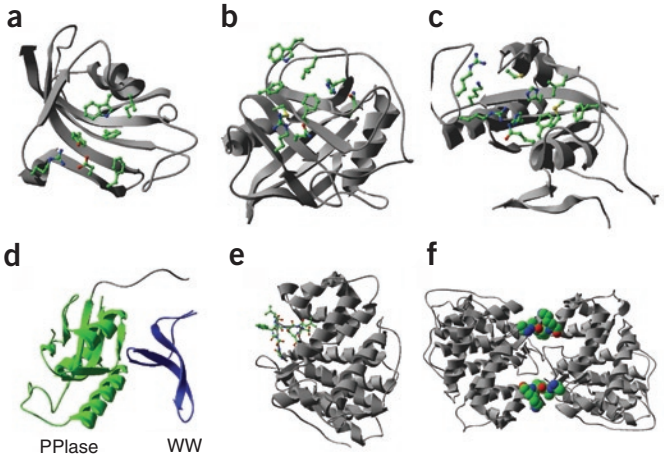

Figure 3 Structural gallery of the four major classes of PPIases. (**a**–**f**) Global folds of PPIase representatives FKBP12 (**a**), CypA (**b**), Pin1 (**c**,**d**) and PTPA (**e**,**f**) are shown[24,38–40]. Even though FKBP12, CypA and Pin1 are structurally unrelated, they share similar active sites (key residues are shown), which suggests the possibility that these seemingly unrelated enzymes may share common features in their catalytic action[45]. A unique feature of Pin1 is the separation of its binding and catalysis functions into distinct domains, WW and PPIase, respectively (**d**). Recent NMR studies suggest communication between the two domains. A ^{15}N backbone dynamics study of Pin1 showed increases in interdomain interactions of varying degrees with addition of different substrates[51]. An NMR study of the regulation of Pin1 isomerase activity by its WW domain suggested a negative regulatory role of the WW domain for multiphosphorylated substrates[52]. Moreover, recent NMR studies of the dynamics of the separate catalytic and WW domains show a possible communication network through the hydrophobic core of the catalytic domain[53] and tuning of WW specificity by loop motions[54]. Distinct from all other known PPIases, the catalytic domain of PTPA is an all-α-helix fold (**e**), and its active site is located at the interface of a substrate-induced dimer (**f**)[40].

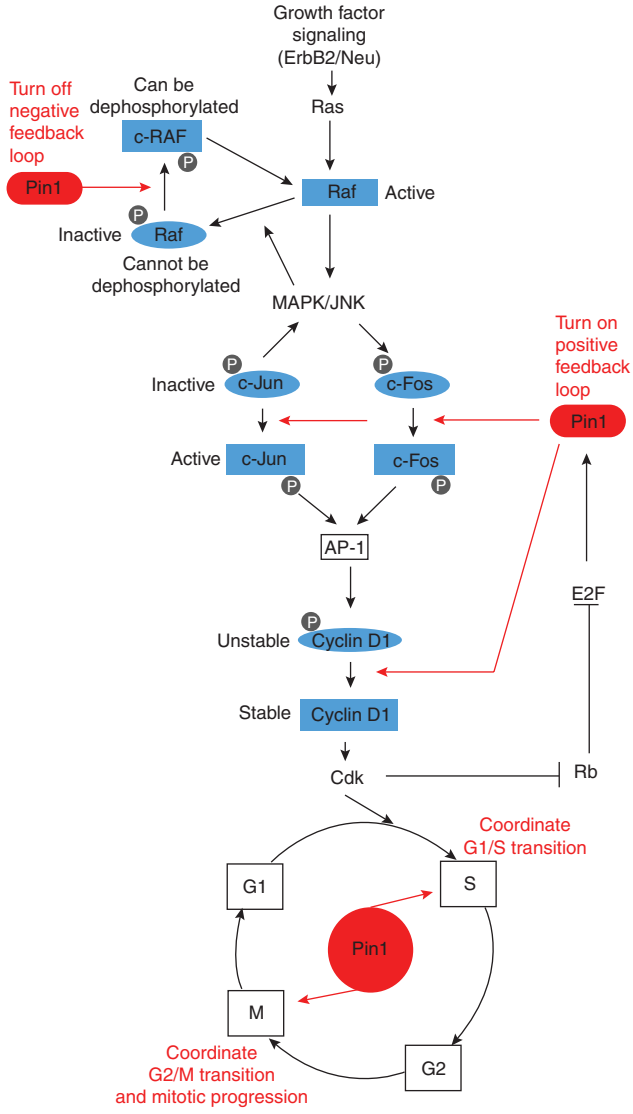

Figure 4 Phosphorylation-dependent prolyl *cis-trans* isomerization as a molecular timer in the Neu-Raf-Ras-MAPK signal pathway. Phosphorylation of proteins on certain Ser/Thr-Pro motifs is an essential signaling mechanism in cell proliferation and transformation in response to growth stimulation such as ErbB2 (Neu) activation. By binding and isomerizing specific pSer/Thr-Pro motifs, Pin1 regulates phosphorylation signaling after phosphorylation. Pin1 activity is tightly regulated normally by multiple mechanisms, including E2F-mediated transcriptional activation in response to growth factor stimulation[72]. Pin1 can target to c-Jun and c-Fos in cooperation with Neu-Ras-MAPK signaling pathways to increase AP-1 transcriptional activity toward its downstream target genes, including the gene encoding cyclin D1 (refs. 64,75). Pin1 can also directly act on cyclin D1 to prevent its nuclear export and ubiquitin-mediated degradation, leading to cyclin D1 stabilization[67], which then in turn activates the E2F transcription factor to enhance Pin1 expression. Therefore, there is a positive feedback loop in which growth stimulation leads to an increase in Pin1 expression, which in turn enhances growth signaling to increase cyclin D1 transcription and stabilization. In addition, Pin1 promotes dephosphorylation of Raf kinase, which is phosphorylated and inactivated by MAP kinases in a negative feedback mechanism, thereby preventing Raf kinase from being inactivated after growth stimulation[74]. Finally, Pin1 can also act on various cell cycle proteins to coordinate progression through the cell cycle. As a result, overexpression of Pin1 enhances the ability of Ras and Neu to transform cells, whereas inhibition or ablation of Pin1 prevents Ras or Neu from inducing tumorigenesis[72,76]. Thus, Pin1 functions as a novel molecular timer of the Neu-Raf-Ras-MAPK signal pathway by acting on multiple targets at various steps of the signal pathway to promote cell proliferation and transformation.

mosome aneuploidy, and tumorigenesis in cell cultures and in transgenic mice[71]. Given that Pin1 is significantly elevated at the G1/S transition[72] and is also subject to cell cycle–specific phosphorylation regulation[50], these results suggest that Pin1 is normally needed for the coordination of the events during the G1/S transition. Interestingly, FKBP12 knockout also results in G1 arrest owing to the overactivity of TGF-β receptor signaling[73], which is normally inhibited by FKBP12 (ref. 22).

Cell signaling. The spatial and temporal regulation of signal transduction is a key mechanism acquired by specific cell types that allows regulated responses of sufficient amplitude and duration to efficiently perform a specific function. The evolution of such regulatory mechanisms, which is often reinforced via positive and negative feedback loops, has allowed for striking specialization in biological systems. A number of studies have demonstrated a critical role for prolyl *cis-trans* isomerization in determining the timing and duration of several signal pathways involved in cell proliferation and transformation.

One of the most well-documented examples is the critical role of phosphorylation-specific prolyl isomerization in amplifying the Neu-Raf-Ras-MAP kinase pathway at multiple levels (**Fig. 4**)[5,7,26]. Upon growth stimulation, growth factor receptors such as ErbB2 (also called Neu) activate Raf and then Ras to turn on the MAP kinase cascade, leading to the activation of MAP kinases. MAP kinases phosphorylate c-Jun and c-fos, thereby enhancing AP-1 transcriptional activity toward its downstream targets such as cyclin D1, which positively promotes cell cycle progression. Furthermore, activation of MAP kinases also leads to phosphorylation of Raf to inactivate Raf in a negative feedback loop[74]. As an E2F downstream target gene, Pin1 orchestrates the regulation of this pathway by directly acting on several intermediaries of this cascade (such as c-Jun, c-fos and cyclin D1) to turn on the positive feedback loop and Raf to turn off the negative feedback loop (**Fig. 4**)[64,67,72,74,75]. Indeed, overexpression of Pin1 enhances the ability of Ras and Neu to transform cells, whereas inhibition or ablation of Pin1 prevents Ras or Neu from inducing cell transformation *in vitro* and cancer development *in vivo*[72,76]. These results indicate that Pin1 controls the duration and outcome of the Neu-Ras signaling pathway.

Further examples of the role of Pin1 in regulation of cell signaling include the activity of Pin1 on the Wnt/β-catenin and cytokine/NF-κB signaling pathways, where Pin1 acts on transcription factors β-catenin and NF-κB (refs. 65,66). β-catenin, a key regulator of cell growth, is activated by mutation of the β-catenin or adenomatous polyposis coli protein (APC) genes or by overexpression[77]. Several studies have confirmed and/or expanded the original findings of Ryo *et al.*, who demonstrated that Pin1 increases the stability of β-catenin by preventing its binding to APC, thereby creating a further positive growth signal for tumor cells[65,78,79]. Furthermore, Pin1 plays a role in the regulation of the NF-κB pathway by inhibiting the interaction of the p65 subunit with IκBα inhibitor after cytokine stimulation, which results in nuclear accumulation of p65 and inhibition of p65 degradation by suppressor of cytokine signaling 1 (SOCS1) (ref. 66). Regardless of the target protein or the exact mechanism of action, it is possible to take a broad view of Pin1 as an orchestrator of certain kinase signaling, where it conducts a troupe of kinases and signaling molecules to keep tempo in a concerted effort to achieve a common goal—for instance, growth promotion or transformation. In other words, by keeping the beat, Pin1 amplifies the music.

The importance of phosphorylation-independent prolyl isomerization in cell signaling has also been documented. The best known exam-

ples are the critical regulatory role for prolyl *cis-trans* isomerization in Itk signaling (**Fig. 5**)[80–83], as described below, and Crk signaling[6,33]. Crk is a member of an adaptor protein family that has several SH2 and SH3 domains. NMR studies have revealed that *cis-trans* isomerization of the prolyl bond at position Gly237-Pro238 (located between two of the SH3 domains) is very slow and that ligand binding to the SH3 domain facilitates an opening of the "gate," where the isomerization rate is increased and the adaptor forms an "open," less restricted active conformation that is catalyzed by CypA (ref. 33). These above findings also point further to the realization that the control of signaling networks is not simply the "right time, right place" but additionally the "correct rate." The emergence of prolyl isomerization as a complementary regulatory mechanism governing such processes suggests that significant further regulation by this mechanism in similar processes awaits discovery.

Gene expression. Prolyl *cis-trans* isomerization has been shown to regulate gene expression by multiple mechanisms. First, Pin1-catalyzed phosphorylation-dependent prolyl isomerization has been shown to directly regulate the function of many transcription factors via many different mechanisms, including modulating the transcriptional activity of c-Jun (ref. 64) and c-fos (ref. 75), modulating protein stability of β-catenin (ref. 65), NF-κB (ref. 66) and c-Myc (ref. 68), or modulating both in the case of p53 (refs. 84–86) and p73 (ref. 87). Pin1 can also regulate general transcription and RNA processing machinery. For example, Pin1 binds to pSer-Pro motifs in the C-terminal domain of the large subunit of RNA polymerase II (CTD) and stimulates CTD dephosphorylation by its phosphatase Fcp1 (refs. 88–90). Recently, it has been reported that Pin1 increases CTD phosphorylation or inhibits CTD dephosphorylation in mammalian cells and might be involved in transcriptional suppression during mitosis[91].

Phosphorylation-independent prolyl *cis-trans* isomerization can also regulate gene expression. Cyp and FKBPs have long been shown to interact with and modulate activities of many transcription factors, including steroid receptors[20] and c-Myb (ref. 92), in which the isomerase activity may be involved in some cases. However, probably the most convincing evidence is in histones. FKBP-family protein Fpr4 interacts with histones H3 and H4 and isomerizes H3P30 and H3P38 in budding yeast[32]. Like Pin1 (ref. 26), Fpr4 contains an N-terminal highly charged nucleolin-like domain that mediates its interaction with histones H3 and H4 and a C-terminal PPIase catalytic domain[32]. The isomerization of H3P38 by Fpr4 inhibits the ability of Set2 methyltransferase to methylate H3K36 and leads to increased transcriptional activation of target genes[32]. Moreover, abrogation of Fpr4 catalytic activity *in vivo* results in increased levels of H3K36 methylation and delayed transcriptional induction kinetics of specific genes in yeast[32]. These results have demonstrated a regulatory role for prolyl *cis-trans* isomerization in gene expression and have provided evidence for crosstalk between histone lysine methylation and prolyl isomerization.

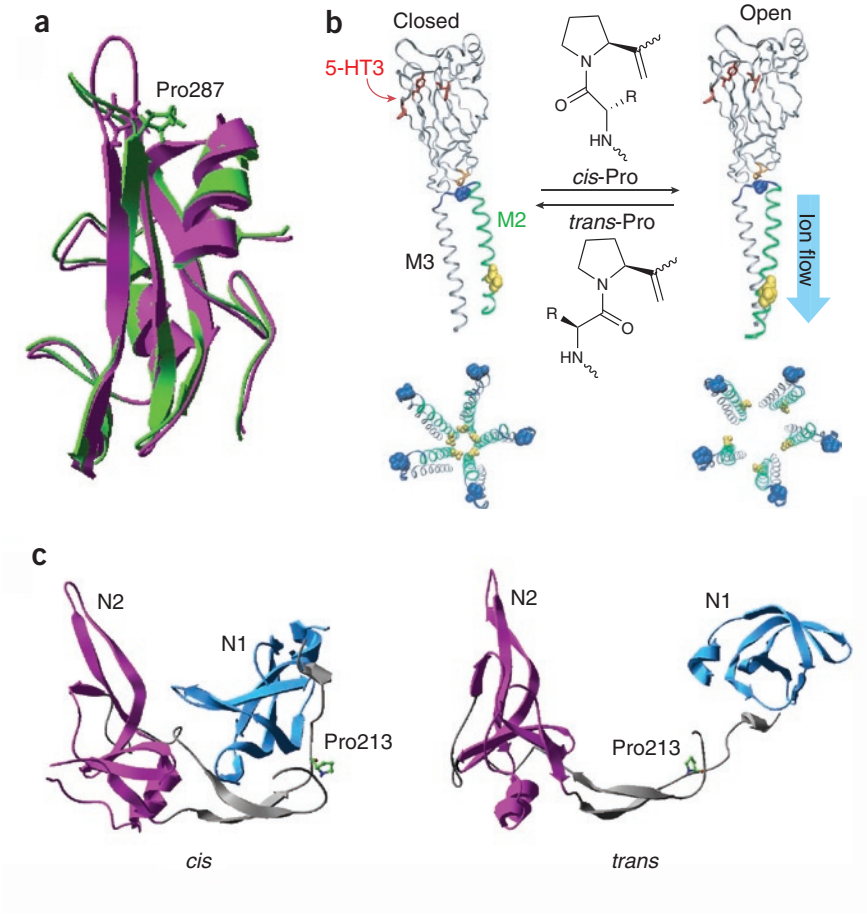

Figure 5 Phosphorylation-independent prolyl *cis-trans* isomerization as a molecular timer in cell signaling, ion channel gating and phage infection. (**a**) In cell signaling protein Itk, Pro287 undergoes CypA-catalyzed *cis-trans* isomerization. The *cis* and *trans* isomer states of Itk adopt different loop structures and regulate the molecular recognition of Itk (refs. 80,81). (**b**) Open and closed forms of the neurotransmitter-gated ion channel 5-HT3 correspond to the *cis* and *trans* isomer states of P*8, a proline residue located in a loop between transmembrane helices M2 and M3 (adapted with permission from Lummis *et al.*[31]). (**c**) The gene-3-protein (G3P) of the filamentous phage fd resides at the tip of the phage and is maintained in a stable but noninfectious state when Pro213 adopts the *cis* conformation. Conversion of Pro213 to the *trans* conformation results in disassembly of G3P into separate N1 and N2 domains and unmasks the TolA binding site in N1, allowing effective binding of G3P to the bacterial F pilus[30,126].

Immune response. The initial interest in prolyl isomerization as a regulatory function in the immune system arose from the discovery that Cyp and FKBP families of PPIases are inhibited by immunosuppressive drugs CsA and FK506 (refs. 8–10). However, surprisingly, inhibition of the enzymatic activities of Cyps and FKBPs is apparently not responsible for the pharmacological efficacy of these drugs[12–16]. Recent reports have shown that prolyl isomerase activity itself may have an important role in the immune response after all. The most well-characterized example is the role for CypA in the regulation of the tyrosine kinase ItK in T-cell activation (**Fig. 5**)[80,81]. CypA forms stable complexes with a proline within the SH2 domain of the kinase in T cells and negatively controls the Itk kinase[83]. Indeed, CypA-null $CD4^+$ T cells are hypersensitive to T-cell receptor (TCR) stimulation, a phenotype consistent with increased activity of Itk (ref. 82). Moreover, mutation of the critical conformationally heterogeneous proline in the SH2 domain of Itk disrupts interaction with CypA

and specifically increases Th2 cytokine production from wild-type CD4+ T cells. These results indicate that CypA-catalyzed *cis-trans* isomerization in Itk plays a critical role in CD4+ T-cell activation[82].

Recent studies have also uncovered the immune function for Pin1, which is not a target for CsA or FK506 (refs. 66,93–96). Upon cytokine stimulation, Pin1 inducibly acts on phosphorylated p65 and prevents NF-κB from being inhibited by its inhibitor IκBα and p65 from being degraded by ubiquitin-mediated proteolysis, which results in nuclear accumulation and activation of NF-κB (ref. 66). Indeed, Pin1-deficient mice and cells are refractory to NF-κB activation by cytokine signals[66]. Furthermore, Pin1 has a critical role in asthma[93]. Pin1 is activated in eosinophils from the airways of people with asthma and is important for both eosinophil survival and granulocyte-macrophage colony-stimulating factor (GM-CSF) production by stabilizing GM-CSF mRNA[93]. Likewise, Pin1 is important for the post-transcriptional regulation of GM-CSF and type 1 inflammatory cytokines by T cells[95]. In addition, Pin1 inhibits the interferon regulatory factor 3 (IRF3)-dependent antiviral response[94]. Upon stimulation of Toll-like receptor 3 (TLR3) or retinoid-inducible gene 1 (RIG1), Pin1 binds to phosphorylated IRF3 and promotes its degradation. Pin1 knockdown or knockout results in sustained double-stranded RNA–induced IRF3-dependent activation and enhanced interferon-β (IFN-β) production, with consequent reduction of virus replication after virus challenge[94]. Notably, CypB interacts with IRF3 and positively regulates dimer formation and activation and cofactor recruitment[97]. These results demonstrate the diversity of function of prolyl isomerases and illustrate the rational evolution of enzyme families capable of both positive and negative regulation of complex biological processes.

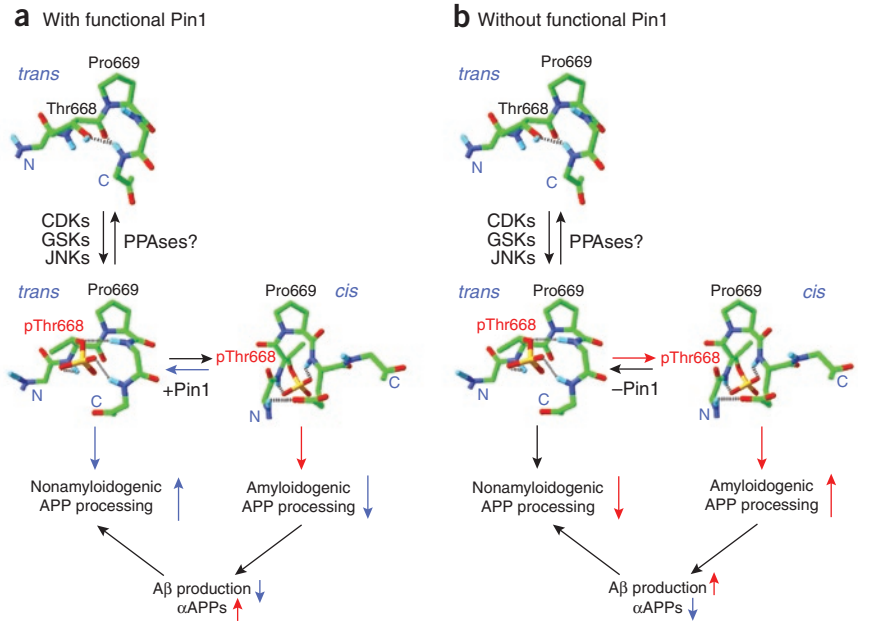

Figure 6 Phosphorylation-dependent prolyl *cis-trans* isomerization as a molecular timer in APP processing and Aβ production in Alzheimer's disease. Phosphorylation of APP on the Thr668-Pro motif occurs during mitosis in the cell cycle and is also increased in Alzheimer's disease brains[108,109]. Before phosphorylation, the Thr668-Pro motif in APP is in *trans* in a helix cap structure[117]. Although the pTh668-Pro motif of APP is likely phosphorylated in *trans* by upstream kinases, it has a tendency to be in *cis*, with the overall content being ~10%, due both to destabilization of the *trans* isomer by loss of hydrogen bonds resulting from a local unfolding of a helix cap and to stabilization of the *cis* isomer by hydrogen bonds involving the phosphate[117]. Pin1 accelerates the *cis-trans* isomerization rate of the APP Thr668-Pro motif by several orders of magnitude over the typical uncatalyzed isomerization rates for pThr-Pro peptides, which results in a dramatic reduction in the average lifetime of both the *cis* (~0.05 s) and *trans* (~0.5 s) isomeric states to fractions of a second[118]. (**a**,**b**) This favors more nonamyloidogenic APP processing, reducing Aβ production (**a**). In contrast, without proper Pin1 function, the *cis* pThr668-Pro motif has a longer lifetime and its population relative to *trans* can be shifted far from its equilibrium value depending on isomer-specific interactions, which might favor more amyloidogenic APP processing and Aβ production (**b**). Therefore, in collaboration with other Alzheimer's disease factors, Pin1 deregulation might promote amyloidogenic APP processing and Aβ production and thus contribute to the pathology of Alzheimer's disease[118]. GSK, glycogen synthase kinase-3; APP, amyloid precursor protein.

Neuronal function. Cyps and FKBPs are enriched far more in the brain than in the immune system and have well-recognized roles in the neuron[98]. For example, correct localization of two of the rhodopsins in the *D. melanogaster* eye requires the chaperone function of the Cyp NinaA (refs. 18,99). FKBP12 forms a complex with the ryanodine and IP3 receptors to regulate their physiological release of intracellular Ca^{2+} (ref. 21). Indeed, FKBP12.6 knockout results in defective ryanodine receptor and heart function in mice[100,101]. Furthermore, CypA protects neurons from death after expression of mutant Cu/Zn superoxide dismutase, which is associated with familial amyotrophic lateral sclerosis[102]. In addition, FK506 and its analogs have profound neuroregenerative and neuroprotective actions independent of its immunosuppressant activity[103]. Even though the importance of PPIase enzymatic activity of Cyps and FKBPs in the neuron is not yet fully understood, prolyl *cis-trans* isomerization has been shown to control the opening (gating) of a neurotransmitter-gated ion channel[31].

5-hydroxytryptamine type 3 (5-HT3) receptors are members of the cysteine-loop receptor superfamily[104]. Neurotransmitter binding in these proteins triggers the opening of an ion channel by inducing conformational change, which has been shown to be prolyl *cis-trans* isomerization (**Fig. 5**)[31]. 5-HT3 receptors have a specific proline (P*8) located at the apex of the loop between the second and third transmembrane helices (M2 and M3). When P*8 is replaced with analogs that favor the *trans* conformation, closed channels are observed. In contrast, unnatural amino acids that have high *cis* preference result in irreversible open channels. These results suggest that neurotransmitter binding may trigger a conformational change that isomerizes P*8 from *trans* to *cis*[31]. Interestingly, proline is often found in the transmembrane regions of ion channels and transporters[105], which suggests an important role for isomerization in regulating the gating of ion channels. Furthermore, because conversion between *cis* and *trans* conformations is rather slow without a catalyst, it remains unknown how neurotransmitters trigger *trans*-to-*cis* isomerization and whether PPIases are involved in the conformational switch.

Phosphorylation-dependent prolyl isomerization has been shown to play a pivotal role in protecting against age-dependent neurodegenerative disorders such as Alzheimer's disease. A growing body of evidence indicates some common features between the normal cell cycle and degenerated Alzheimer's disease neurons, especially mitotic

phosphorylation on certain Ser/Thr-Pro motifs[106,107]. Of significance is the incorporation of these mitotic MPM-2 phosphoepitopes into tangles and their appearance before tangle formation and neurodegeneration in Alzheimer's disease brains. Phosphorylation of amyloid precursor protein (APP) on the Thr668-Pro motif, which occurs in mitotic cells[108], is also increased in Alzheimer's disease brains and can elevate Aβ secretion *in vitro*[109]. Thus, increased proline-directed phosphorylation is a common feature in both tangle and plaque pathologies, pointing to a possible role for Pin1.

Pin1 is mainly expressed in most neurons at unusually high levels, and its expression is induced during neuronal differentiation[23,64,65,110–112]. However, in Alzheimer's disease brains, Pin1 colocalizes with the neurofibrillary tangles, which results in depletion of soluble Pin1 (ref. 110). Furthermore, Pin1 is inactivated by oxidative modifications in Alzheimer's disease brains, even at early stages of the disease[27,113]. Moreover, Pin1 promoter polymorphisms are associated with reduced Pin1 levels in blood cells and with an increased risk for Alzheimer's disease in some studies[114], although not in others[115]. Thus, Pin1 is downregulated or inactivated by various mechanisms in Alzheimer's disease.

The significance of such Pin1 inhibition in Alzheimer's disease is further underscored by the findings that Pin1 expression inversely correlates with the predicted neuronal vulnerability in normally aged brains and also with actual neurofibrillary degeneration in Alzheimer's disease[116]. Furthermore, Pin1 regulates the biological function, dephosphorylation and/or processing of mitotic phosphoproteins, including tau and APP (refs. 49,90,91,110). Pin1 binds to pThr231-tau and restores its ability to bind microtubules and promote microtubule assembly[110]. Furthermore, Pin1 also facilitates tau dephosphorylation by PP2A owing to the conformational specificity of PP2A (refs. 49,112). In the case of APP, after Thr668 phosphorylation, a distinct new *cis* population appears and exchanges very slowly with the *trans* conformation (**Fig. 6**)[117]. Furthermore, Pin1 has been shown to act on the pThr668-Pro motif in full-length APP (ref. 118), in contrast to a recent report that Pin1 does not act on full-length protein[119]. More importantly, Pin1 greatly accelerates the pThr668-Pro *cis-trans* isomerization, thereby regulating the APP intracellular domain between two conformations as visualized by NMR (ref. 118). Moreover, Pin1 can promote nonamyloidogenic APP processing, thereby producing neurotrophic αAPPs and reducing neurotoxic Aβ peptides (**Fig. 6**)[118]. These results suggest that Pin1 may be neuroprotective against age-dependent neurodegeneration in Alzheimer's disease[106].

Indeed, Pin1 knockout mice develop progressive age-dependent neuropathy characterized by motor and behavioral deficits, tau- and Aβ-related pathologies and neuronal degeneration, which resembles many aspects of human Alzheimer's disease[116,118]. In contrast to most other Alzheimer's disease mouse models, in which overexpression of one or several genes is used to induce certain Alzheimer's disease phenotypes[120,121], Pin1 knockout mice are the first such model based on a single gene deletion[116,118]. In addition, Pin1 acts on the scaffolding protein gephyrin and is crucial for the proper functioning of glycinergic synapses[122]. Moreover, Pin1 has been shown to protect against spinal cord injury by preventing JNK3-induced Mcl-1 degradation, cytochrome *c* release, and apoptosis *in vitro* and *in vivo*[123]. Thus, Pin1 has an important neuroprotective function and may be a potential new therapeutic target for Alzheimer's disease.

Infection. A growing body of evidence indicates that microorganisms can also use prolyl *cis-trans* isomerization as a regulatory mechanism to gain entry into host cells. For example, CypA is required for full infectivity of HIV-1, which is intriguing in that this demonstrates that HIV-1 has co-opted a host cell factor to facilitate efficient viral infection[19,124]. The mechanism by which HIV-1 gains entry to a host cell is by means of interaction with CypA. Specifically, CypA binds to an exposed proline-rich loop in the HIV-1 capsid protein A (CA) domain of HIV-1 Gag envelope protein. Mutational inactivation of the CypA binding site or inhibition of the CA-CypA interaction by CsA inhibits replication of most HIV-1 strains, and HIV-1 replication is attenuated in a human T-cell line that lacks CypA. In this case, it seems that the function of the Cyp-CA interaction is to block recognition of the HIV-1 virion by the host cell restriction factor Ref1, an intrinsic antiviral cellular protection mechanism[41,125]. The recruitment of a normal host factor by a virus such as HIV-1 to evade detection by host restriction factors and thereby increase infectivity represents a novel mechanism of parasitism, although the exact regulatory function of CypA is not fully understood.

The unusual chemical property of proline has been shown as a molecular timer for bacterial infection by the filamentous phage fd (**Fig. 5**)[30,126]. Specifically, the phage tip protein G3P, a key mediator of infectivity, is directly controlled by isomerization of a key residue: Pro213. Interestingly, G3P, which is anchored to the phage tip by its C-terminal domain, interacts directly with the bacterial F pilus via the G3P N-terminal domain N1, which in turn exposes the binding site for TolA, the phage receptor at the bacterial cell surface. This domain opening serves as a timer for phage infection, where Pro213 in the *cis* conformation serves to keep the timer turned off in a stable but not infectious phage state. However, the timer is turned on when the second N-terminal domain of G3P binds to the bacterial F pilus, thus relieving the conformational tension that keeps Pro213 in the *cis* state. Consequently, the conformational change of Pro213 from *cis* to *trans* sets the phage infection timer to 'on', and infection, which is mediated by TolA binding, begins[30]. In addition, mutational analysis indicates that the local sequence around Pro213 ensures that the *cis*-to-*trans* conversion is appropriately slow, and therefore the infection lifetime is sufficient for efficient infection[30]. The development of an extracellular proline 'switch' functioning without an apparent catalytic enzyme adds further to our knowledge of the broad use of the isomerization phenomenon in nature and suggests that it is important to examine this further in such contexts as cell-cell communication and antigen presentation.

Conclusion and future challenges

The ubiquitous presence of PPIases and the breadth of their biological roles underscore the importance of prolyl *cis-trans* isomerization as a fundamental molecular switch that controls the timing of key biological processes. The *cis* and *trans* isomers provide stable local motifs that differ dramatically in structure, thereby providing a mechanism for selecting distinct pools of binding partners even in the context of otherwise unstructured regions of proteins. The intrinsically slow interconversion between *cis* and *trans* isoforms further provides the opportunity to regulate the timing of exchange through enzymatic acceleration by specific PPIases. An added level of regulation is provided by phosphorylation-dependent PPIase activity. Examples of such molecular switches elucidated thus far demonstrate both the utility of prolyl *cis-trans* isomerization as a key rate-limiting step and the regulatory power of PPIases in controlling the timing of cellular processes.

Some major challenges for the future include (i) defining the structural and functional differences of proteins that adopt *cis* and *trans* conformations, (ii) developing tools to visualize these conformational changes, (iii) determining how these conformations are regulated and (iv) correlating these different conformations with protein functions. It is also important to determine how this mechanism is used in conjunction with many other regulatory mechanisms known to keep signaling molecules in check until the proper time and place, and to elucidate the

role of deregulated prolyl *cis-trans* isomerization in the pathology of human diseases. Finally, given the availability of Cyp and FKBP inhibitors such as CsA, FK506 and rapamycin, and with the active development of Pin1 inhibitors, it would be beneficial to explore whether this new mechanism can be used as a drug target for treating certain human diseases.

Looking to the future, cellular dynamics is an emerging scientific frontier that requires synergistic coupling of studies that span atomic to cellular levels. How does the 'readout', or some experimentally measurable outcome of a biological process, depend on the rate of a given step in the process? Prolyl *cis-trans* isomerization is a particularly effective bridge connecting a kinetically and thermodynamically characterized molecular switch to a measurable cellular output. Further studies that correlate cellular outputs with the effects of 'tuning' this switching process will potentially advance our understanding of the exquisite choreography of biological processes that constitute life.

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COMPETING INTERESTS STATEMENT

The authors declare no competing financial interests.

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1. Yaffe, M.B. *et al.* Sequence-specific and phosphorylation-dependent proline isomerization: a potential mitotic regulatory mechanism. *Science* **278**, 1957–1960 (1997).
2. Zhou, X.Z., Lu, P.J., Wulf, G. & Lu, K.P. Phosphorylation-dependent prolyl isomerization: a novel signaling regulatory mechanism. *Cell. Mol. Life Sci.* **56**, 788–806 (1999).
3. Fischer, G. & Aumuller, T. Regulation of peptide bond cis/transisomerization by enzyme catalysis and its implication in physiological processes. *Rev. Physiol. Biochem. Pharmacol.* **148**, 105–150 (2003).
4. Andreotti, A.H. Native state proline isomerization: an intrinsic molecular switch. *Biochemistry* **42**, 9515–9524 (2003).
5. Wulf, G., Finn, G., Suizu, F. & Lu, K.P. Phosphorylation-specific prolyl isomerization: is there an underlying theme? *Nat. Cell Biol.* **7**, 435–441 (2005).
6. Nicholson, L.K. & Lu, K.P. Prolyl cis-trans isomerization as a molecular timer in Crk signaling. *Mol. Cell* **25**, 483–485 (2007).
7. Lu, K.P. & Zhou, X.Z. The prolyl isomerase Pin1: a pivotal new twist in phosphorylation signalling and human disease. *Nat. Rev. Mol. Cell Biol.* (in the press).
8. Harding, M.W., Galat, A., Uehling, D.E. & Schreiber, S.L. A receptor for the immunosuppressant FK506 is a cis-trans peptidyl-prolyl isomerase. *Nature* **341**, 758–760 (1989).
9. Fischer, G., Wittmann-Liebold, B., Lang, K., Kiefhaber, T. & Schmid, F.X. Cyclophilin and peptidyl-prolyl cis-trans isomerase are probably identical proteins. *Nature* **337**, 476–478 (1989).
10. Takahashi, N., Hayano, T. & Suzuki, M. Peptidyl-prolyl cis-trans isomerase is the cyclosporin A-binding protein cyclophilin. *Nature* **337**, 473–475 (1989).
11. Siekierka, J.J., Hung, S.H., Poe, M., Lin, C.S. & Sigal, N.H. A cytosolic binding protein for the immunosuppressant FK506 has peptidyl-prolyl isomerase activity but is distinct from cyclophilin. *Nature* **341**, 755–757 (1989).
12. Liu, J. *et al.* Calcineurin is a common target of cyclophilin-cyclosporin A and FKBP-FK506 complexes. *Cell* **66**, 807–815 (1991).
13. Heitman, J., Movva, N.R. & Hall, M.N. Targets for cell cycle arrest by the immunosuppressant rapamycin in yeast. *Science* **253**, 905–909 (1991).
14. Schreiber, S.L. & Crabtree, G.R. The mechanism of action of cyclosporin A and FK506. *Immunol. Today* **13**, 136–142 (1992).
15. Heitman, J., Movva, N.R. & Hall, M.N. Proline isomerases at the crossroads of protein folding, signal transduction, and immunosuppression. *New Biol.* **4**, 448–460 (1992).
16. Hunter, T. Prolyl isomerase and nuclear function. *Cell* **92**, 141–143 (1998).
17. Dolinski, K., Muir, S., Cardenas, M. & Heitman, J. All cyclophilins and FKBPs are dispensible for viability in *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci. USA* **94**, 13093–13098 (1997).
18. Shieh, B.H., Stamnes, M.A., Seavello, S., Harris, G.L. & Zuker, C.S. The ninaA gene required for visual transduction in *Drosophila* encodes a homologue of cyclosporin A-binding protein. *Nature* **338**, 67–70 (1989).
19. Thali, M. *et al.* Functional association of cyclophilin A with HIV-1 virions. *Nature* **372**, 363–365 (1994).
20. Tai, P.K., Albers, M.W., Chang, H., Faber, L.E. & Schreiber, S.L. Association of a 59-kilodalton immunophilin with the glucocorticoid receptor complex. *Science* **256**, 1315–1318 (1992).
21. Brillantes, A.B. *et al.* Stabilization of calcium release channel (ryanodine receptor) function by FK506-binding protein. *Cell* **77**, 513–523 (1994).
22. Wang, T. *et al.* The immunophilin FKBP12 functions as a common inhibitor of the TGF beta family type I receptors. *Cell* **86**, 435–444 (1996).
23. Lu, K.P., Hanes, S.D. & Hunter, T. A human peptidyl-prolyl isomerase essential for regulation of mitosis. *Nature* **380**, 544–547 (1996).
24. Ranganathan, R., Lu, K.P., Hunter, T. & Noel, J.P. Structural and functional analysis of the mitotic peptidyl-prolyl isomerase Pin1 suggests that substrate recognition is phosphorylation dependent. *Cell* **89**, 875–886 (1997).
25. Lu, P.J., Zhou, X.Z., Shen, M. & Lu, K.P. A function of WW domains as phosphoserine- or phosphothreonine-binding modules. *Science* **283**, 1325–1328 (1999).
26. Lu, K.P. Pinning down cell signaling, cancer and Alzheimer's disease. *Trends Biochem. Sci.* **29**, 200–209 (2004).
27. Butterfield, D.A. *et al.* Pin1 in Alzheimer's disease. *J. Neurochem.* **98**, 1697–1706 (2006).
28. Maudsley, S. & Mattson, M.P. Protein twists and turns in Alzheimer disease. *Nat. Med.* **12**, 392–393 (2006).
29. Yeh, E.S. & Means, A.R. Pin1, the cell cycle and cancer. *Nat. Rev. Cancer* **7**, 381–388 (2007).
30. Eckert, B., Martin, A., Balbach, J. & Schmid, F.X. Prolyl isomerization as a molecular timer in phage infection. *Nat. Struct. Mol. Biol.* **12**, 619–623 (2005).
31. Lummis, S.C. *et al.* Cis-trans isomerization at a proline opens the pore of a neurotransmitter-gated ion channel. *Nature* **438**, 248–252 (2005).
32. Nelson, C.J., Santos-Rosa, H. & Kouzarides, T. Proline isomerization of histone H3 regulates lysine methylation and gene expression. *Cell* **126**, 905–916 (2006).
33. Sarkar, P., Reichman, C., Saleh, T., Birge, R.B. & Kalodimos, C.G. Proline cis-trans isomerization controls autoinhibition of a signaling protein. *Mol. Cell* **25**, 413–426 (2007).
34. Stewart, D.E., Sarkar, A. & Wampler, J.E. Occurrence and role of cis peptide bonds in protein structures. *J. Mol. Biol.* **214**, 253–260 (1990).
35. Pal, D. & Chakrabarti, P. Cis peptide bonds in proteins: residues involved, their conformations, interactions and locations. *J. Mol. Biol.* **294**, 271–288 (1999).
36. Pahlke, D., Leitner, D., Wiedemann, U. & Labudde, D. COPS–cis/trans peptide bond conformation prediction of amino acids on the basis of secondary structure information. *Bioinformatics* **21**, 685–686 (2005).
37. Pahlke, D., Freund, C., Leitner, D. & Labudde, D. Statistically significant dependence of the Xaa-Pro peptide bond conformation on secondary structure and amino acid sequence. *BMC Struct. Biol.* **5**, 8 (2005).
38. Kallen, J. *et al.* Structure of human cyclophilin and its binding site for cyclosporin A determined by X-ray crystallography and NMR spectroscopy. *Nature* **353**, 276–279 (1991).
39. Van Duyne, G.D., Standaert, R.F., Karplus, P.A., Schreiber, S.L. & Clardy, J. Atomic structure of FKBP-FK506, an immunophilin-immunosuppressant complex. *Science* **252**, 839–842 (1991).
40. Leulliot, N. *et al.* Crystal structure of the PP2A phosphatase activator: implications for its PP2A-specific PPIase activity. *Mol. Cell* **23**, 413–424 (2006).
41. Howard, B.R., Vajdos, F.F., Li, S., Sundquist, W.I. & Hill, C.P. Structural insights into the catalytic mechanism of cyclophilin A. *Nat. Struct. Biol.* **10**, 475–481 (2003).
42. Eisenmesser, E.Z., Bosco, D.A., Akke, M. & Kern, D. Enzyme dynamics during catalysis. *Science* **295**, 1520–1523 (2002).
43. Trzesniak, D. & van Gunsteren, W.F. Catalytic mechanism of cyclophilin as observed in molecular dynamics simulations: pathway prediction and reconciliation of X-ray crystallographic and NMR solution data. *Protein Sci.* **15**, 2544–2551 (2006).
44. Labeikovsky, W., Eisenmesser, E.Z., Bosco, D.A. & Kern, D. Structure and dynamics of Pin1 during catalysis by NMR. *J. Mol. Biol.* **367**, 1370–1381 (2007).
45. Fanghanel, J. & Fischer, G. Insights into the catalytic mechanism of peptidyl prolyl cis/trans isomerases. *Front. Biosci.* **9**, 3453–3478 (2004).
46. Fersht, A. *Structure and Mechanism in Protein Science: a Guide to Enzyme Catalysis and Protein Folding* (WH Freeman, New York, 1999).
47. Weiwad, M., Kullertz, G., Schutkowski, M. & Fischer, G. Evidence that the substrate backbone conformation is critical to phosphorylation by p42 MAP kinase. *FEBS Lett.* **478**, 39–42 (2000).
48. Brown, N.R., Noble, M.E., Endicott, J.A. & Johnson, L.N. The structural basis for specificity of substrate and recruitment peptides for cyclin-dependent kinases. *Nat. Cell Biol.* **1**, 438–443 (1999).
49. Zhou, X.Z. *et al.* Pin1-dependent prolyl isomerization regulates dephosphorylation of Cdc25C and tau proteins. *Mol. Cell* **6**, 873–883 (2000).
50. Lu, P.J., Zhou, X.Z., Liou, Y.C., Noel, J.P. & Lu, K.P. Critical role of WW domain phosphorylation in regulating its phosphoserine-binding activity and the Pin1 function. *J. Biol. Chem.* **277**, 2381–2384 (2002).
51. Jacobs, D.M. *et al.* Peptide binding induces large scale changes in inter-domain mobility in human Pin1. *J. Biol. Chem.* **278**, 26174–26182 (2003).
52. Smet, C., Wieruszeski, J.M., Buee, L., Landrieu, I. & Lippens, G. Regulation of Pin1 peptidyl-prolyl cis/trans isomerase activity by its WW binding module on a multi-phosphorylated peptide of Tau protein. *FEBS Lett.* **579**, 4159–4164 (2005).
53. Namanja, A.T. *et al.* Substrate recognition reduces side-chain flexibility for conserved hydrophobic residues in human Pin1. *Structure* **15**, 313–327 (2007).
54. Peng, T., Zintsmaster, J.S., Namanja, A.T. & Peng, J.W. Sequence-specific dynamics modulate recognition specificity in WW domains. *Nat. Struct. Mol. Biol.* **14**, 325–331 (2007).

55. Nurse, P. Cyclin dependent kinases and cell cycle control (Nobel lecture). *ChemBioChem* **3**, 596–603 (2002).
56. Ubersax, J.A. *et al.* Targets of the cyclin-dependent kinase Cdk1. *Nature* **425**, 859–864 (2003).
57. Winkler, K.E., Swenson, K.I., Kornbluth, S. & Means, A.R. Requirement of the prolyl isomerase Pin1 for the replication checkpoint. *Science* **287**, 1644–1647 (2000).
58. Stukenberg, P.T. & Kirschner, M.W. Pin1 acts catalytically to promote a conformational change in Cdc25. *Mol. Cell* **7**, 1071–1083 (2001).
59. Shen, M., Stukenberg, P.T., Kirschner, M.W. & Lu, K.P. The essential mitotic peptidyl-prolyl isomerase Pin1 binds and regulates mitosis-specific phosphoproteins. *Genes Dev.* **12**, 706–720 (1998).
60. Crenshaw, D.G., Yang, J., Means, A.R. & Kornbluth, S. The mitotic peptidyl-prolyl isomerase, Pin1, interacts with Cdc25 and Plx1. *EMBO J.* **17**, 1315–1327 (1998).
61. Okamoto, K. & Sagata, N. Mechanism for inactivation of the mitotic inhibitory kinase Wee1 at M phase. *Proc. Natl. Acad. Sci. USA* **104**, 3753–3758 (2007).
62. Bernis, C. *et al.* Pin1 stabilizes Emi1 during G2 phase by preventing its association with SCF(betatrcp). *EMBO Rep.* **8**, 91–98 (2007).
63. Xu, Y.X. & Manley, J.L. The prolyl isomerase Pin1 functions in mitotic chromosome condensation. *Mol. Cell* **26**, 287–300 (2007).
64. Wulf, G.M. *et al.* Pin1 is overexpressed in breast cancer and potentiates the transcriptional activity of phosphorylated c-Jun towards the cyclin D1 gene. *EMBO J.* **20**, 3459–3472 (2001).
65. Ryo, A., Nakamura, N., Wulf, G., Liou, Y.C. & Lu, K.P. Pin1 regulates turnover and subcellular localization of beta-catenin by inhibiting its interaction with APC. *Nat. Cell Biol.* **3**, 793–801 (2001).
66. Ryo, A. *et al.* Regulation of NF-kappaB signaling by Pin1-dependent prolyl isomerization and ubiquitin-mediated proteolysis of p65/RelA. *Mol. Cell* **12**, 1413–1426 (2003).
67. Liou, Y.C. *et al.* Loss of Pin1 function in the mouse causes phenotypes resembling cyclin D1-null phenotypes. *Proc. Natl. Acad. Sci. USA* **99**, 1335–1340 (2002).
68. Yeh, E. *et al.* A signalling pathway controlling c-Myc degradation that impacts oncogenic transformation of human cells. *Nat. Cell Biol.* **6**, 308–318 (2004).
69. van Drogen, F. *et al.* Ubiquitylation of cyclin E requires the sequential function of SCF complexes containing distinct hCdc4 isoforms. *Mol. Cell* **23**, 37–48 (2006).
70. Fujimori, F., Takahashi, K., Uchida, C. & Uchida, T. Mice lacking Pin1 develop normally, but are defective in entering cell cycle from G(0) arrest. *Biochem. Biophys. Res. Commun.* **265**, 658–663 (1999).
71. Suizu, F., Ryo, A., Wulf, G., Lim, J. & Lu, K.P. Pin1 regulates centrosome duplication and its overexpression induces centrosome amplification, chromosome instability and oncogenesis. *Mol. Cell. Biol.* **26**, 1463–1479 (2006).
72. Ryo, A. *et al.* Pin1 is an E2F target gene essential for the Neu/Ras-induced transformation of mammary epithelial cells. *Mol. Cell. Biol.* **22**, 5281–5295 (2002).
73. Aghdasi, B. *et al.* FKBP12, the 12-kDa FK506-binding protein, is a physiologic regulator of the cell cycle. *Proc. Natl. Acad. Sci. USA* **98**, 2425–2430 (2001).
74. Dougherty, M.K. *et al.* Regulation of Raf-1 by direct feedback phosphorylation. *Mol. Cell* **17**, 215–224 (2005).
75. Monje, P., Hernandez-Losa, J., Lyons, R.J., Castellone, M.D. & Gutkind, J.S. Regulation of the transcriptional activity of c-Fos by ERK. A novel role for the prolyl isomerase Pin1. *J. Biol. Chem.* **280**, 35081–35084 (2005).
76. Wulf, G., Garg, P., Liou, Y.C., Iglehart, D. & Lu, K.P. Modeling breast cancer *in vivo* and *ex vivo* reveals an essential role of Pin1 in tumorigenesis. *EMBO J.* **23**, 3397–3407 (2004).
77. Moon, R.T., Bowerman, B., Boutros, M. & Perrimon, N. The promise and perils of Wnt signaling through beta-catenin. *Science* **296**, 1644–1646 (2002).
78. Pang, R. *et al.* Pin1 overexpression and beta-catenin gene mutations are distinct oncogenic events in human hepatocellular carcinoma. *Oncogene* **23**, 4182–4186 (2004).
79. Chen, S.Y. *et al.* Activation of beta-catenin signaling in prostate cancer by peptidyl-prolyl isomerase Pin1-mediated abrogation of the androgen receptor-beta-catenin interaction. *Mol. Cell. Biol.* **26**, 929–939 (2006).
80. Mallis, R.J., Brazin, K.N., Fulton, D.B. & Andreotti, A.H. Structural characterization of a proline-driven conformational switch within the Itk SH2 domain. *Nat. Struct. Biol.* **9**, 900–905 (2002).
81. Brazin, K.N., Mallis, R.J., Fulton, D.B. & Andreotti, A.H. Regulation of the tyrosine kinase Itk by the peptidyl-prolyl isomerase cyclophilin A. *Proc. Natl. Acad. Sci. USA* **99**, 1899–1904 (2002).
82. Colgan, J. *et al.* Cyclophilin A regulates TCR signal strength in CD4+ T cells via a proline-directed conformational switch in Itk. *Immunity* **21**, 189–201 (2004).
83. Pletneva, E.V., Sundd, M., Fulton, D.B. & Andreotti, A.H. Molecular details of Itk activation by prolyl isomerization and phospholigand binding: the NMR structure of the Itk SH2 domain bound to a phosphopeptide. *J. Mol. Biol.* **357**, 550–561 (2006).
84. Zheng, H. *et al.* The prolyl isomerase Pin1 is a regulator of p53 in genotoxic response. *Nature* **419**, 849–853 (2002).
85. Zacchi, P. *et al.* The prolyl isomerase Pin1 reveals a mechanism to control p53 functions after genotoxic insults. *Nature* **419**, 853–857 (2002).
86. Wulf, G.M., Liou, Y.C., Ryo, A., Lee, S.W. & Lu, K.P. Role of Pin1 in the regulation of p53 stability and p21 transactivation, and cell cycle checkpoints in response to DNA damage. *J. Biol. Chem.* **277**, 47976–47979 (2002).
87. Mantovani, F. *et al.* Pin1 links the activities of c-Abl and p300 in regulating p73 function. *Mol. Cell* **14**, 625–636 (2004).
88. Verdecia, M.A., Bowman, M.E., Lu, K.P., Hunter, T. & Noel, J.P. Structural basis for phosphoserine-proline recognition by group IV WW domains. *Nat. Struct. Biol.* **7**, 639–643 (2000).
89. Wu, X. *et al.* The Ess1 prolyl isomerase is linked to chromatin remodeling complexes and the general transcription machinery. *EMBO J.* **19**, 3727–3738 (2000).
90. Kops, O., Zhou, X.Z. & Lu, K.P. Pin1 enhances the dephosphorylation of the C-terminal domain of the RNA polymerase II by Fcp1. *FEBS Lett.* **513**, 305–311 (2002).
91. Xu, Y.X., Hirose, Y., Zhou, X.Z., Lu, K.P. & Manley, J.L. Pin1 modulates the structure and function of human RNA polymerase II. *Genes Dev.* **17**, 2765–2776 (2003).
92. Leverson, J.D. & Ness, S.A. Point mutations in v-Myb disrupt a cyclophilin-catalyzed negative regulatory mechanism. *Mol. Cell* **1**, 203–211 (1998).
93. Shen, Z.J., Esnault, S. & Malter, J.S. The peptidyl-prolyl isomerase Pin1 regulates the stability of granulocyte-macrophage colony-stimulating factor mRNA in activated eosinophils. *Nat. Immunol.* **6**, 1280–1287 (2005).
94. Saitoh, T. *et al.* Negative regulation of interferon-regulatory factor 3-dependent innate antiviral response by the prolyl isomerase Pin1. *Nat. Immunol.* **7**, 598–605 (2006).
95. Esnault, S. *et al.* Pin1 modulates the type 1 immune response. *PLoS ONE* **2**, e226 (2007).
96. Yu, L. *et al.* Regulation of Bruton's tyrosine kinase (Btk) by the peptidyl-prolyl isomerase Pin1. *J. Biol. Chem.* **281**, 18201–18207 (2006).
97. Obata, Y. *et al.* Role of cyclophilin B in activation of interferon regulatory factor-3. *J. Biol. Chem.* **280**, 18355–18360 (2005).
98. Snyder, S.H., Lai, M.M. & Burnett, P.E. Immunophilins in the nervous system. *Neuron* **21**, 283–294 (1998).
99. Baker, E.K., Colley, N.J. & Zuker, C.S. The cyclophilin homolog NinaA functions as a chaperone, forming a stable complex *in vivo* with its protein target rhodopsin. *EMBO J.* **13**, 4886–4895 (1994).
100. Xin, H.B. *et al.* Oestrogen protects FKBP12.6 null mice from cardiac hypertrophy. *Nature* **416**, 334–338 (2002).
101. Wehrens, X.H. *et al.* FKBP12.6 deficiency and defective calcium release channel (ryanodine receptor) function linked to exercise-induced sudden cardiac death. *Cell* **113**, 829–840 (2003).
102. Lee, J.P. *et al.* The role of immunophilins in mutant superoxide dismutase-1linked familial amyotrophic lateral sclerosis. *Proc. Natl. Acad. Sci. USA* **96**, 3251–3256 (1999).
103. Snyder, S.H. *et al.* Neural actions of immunophilin ligands. *Trends Pharmacol. Sci.* **19**, 21–26 (1998).
104. Lester, H.A., Dibas, M.I., Dahan, D.S., Leite, J.F. & Dougherty, D.A. Cys-loop receptors: new twists and turns. *Trends Neurosci.* **27**, 329–336 (2004).
105. Sansom, M.S. & Weinstein, H. Hinges, swivels and switches: the role of prolines in signalling via transmembrane alpha-helices. *Trends Pharmacol. Sci.* **21**, 445–451 (2000).
106. Lu, K.P., Liou, Y.C. & Vincent, I. Proline-directed phosphorylation and isomerization in mitotic regulation and in Alzheimer's disease. *Bioessays* **25**, 174–181 (2003).
107. Yang, Y. & Herrup, K. Cell division in the CNS: protective response or lethal event in post-mitotic neurons? *Biochim. Biophys. Acta* **1772**, 457–466 (2007).
108. Suzuki, T. *et al.* Cell cycle-dependent regulation of the phosphorylation and metabolism of the Alzheimer amyloid precursor protein. *EMBO J.* **13**, 1114–1122 (1994).
109. Lee, M.S. *et al.* APP processing is regulated by cytoplasmic phosphorylation. *J. Cell Biol.* **163**, 83–95 (2003).
110. Lu, P.J., Wulf, G., Zhou, X.Z., Davies, P. & Lu, K.P. The prolyl isomerase Pin1 restores the function of Alzheimer-associated phosphorylated tau protein. *Nature* **399**, 784–788 (1999).
111. Thorpe, J.R. *et al.* Shortfalls in the peptidyl-prolyl cis-trans isomerase protein Pin1 in neurons are associated with frontotemporal dementias. *Neurobiol. Dis.* **17**, 237–249 (2004).
112. Hamdane, M. *et al.* Pin1 allows for differential Tau dephosphorylation in neuronal cells. *Mol. Cell. Neurosci.* **32**, 155–160 (2006).
113. Sultana, R. *et al.* Oxidative modification and down-regulation of Pin1 in Alzheimer's disease hippocampus: a redox proteomics analysis. *Neurobiol. Aging* **27**, 918–925 (2006).
114. Segat, L. *et al.* Pin1 promoter polymorphisms are associated with Alzheimer's disease. *Neurobiol. Aging* **28**, 69–74 (2007).
115. Nowotny, P. *et al.* Association studies between common variants in prolyl isomerase Pin1 and the risk for late-onset Alzheimer's disease. *Neurosci. Lett.* **419**, 15–17 (2007).
116. Liou, Y.-C. *et al.* Role of the prolyl isomerase Pin1 in protecting against age-dependent neurodegeneration. *Nature* **424**, 556–561 (2003).
117. Ramelot, T.A. & Nicholson, L.K. Phosphorylation-induced structural changes in the amyloid precursor protein cytoplasmic tail detected by NMR. *J. Mol. Biol.* **307**, 871–884 (2001).
118. Pastorino, L. *et al.* The prolyl isomerase Pin1 regulates amyloid precursor protein processing and amyloid-beta production. *Nature* **440**, 528–534 (2006).
119. Akiyama, H., Shin, R.W., Uchida, C., Kitamoto, T. & Uchida, T. Pin1 promotes production of Alzheimer's amyloid beta from beta-cleaved amyloid precursor protein. *Biochem. Biophys. Res. Commun.* **336**, 521–529 (2005).
120. Wong, P.C., Cai, H., Borchelt, D.R. & Price, D.L. Genetically engineered mouse models of neurodegenerative diseases. *Nat. Neurosci.* **5**, 633–639 (2002).
121. Geschwind, D.H. Tau phosphorylation, tangles, and neurodegeneration: the chicken or the egg. *Neuron* **40**, 457–460 (2003).
122. Zita, M.M. *et al.* Post-phosphorylation prolyl isomerisation of gephyrin represents a mechanism to modulate glycine receptors function. *EMBO J.* **26**, 1761–1771 (2007).
123. Li, Q.M. *et al.* Activation of JNK3 after injury induces cytochrome C release by facilitating Pin1-dependent degradation of Mcl-1. *J. Neurosci.* **27**, 8395–8404 (2007).
124. Braaten, D., Franke, E.K. & Luban, J. Cyclophilin A is required for an early step in the

life cycle of human immunodeficiency virus type 1 before the initiation of reverse transcription. *J. Virol.* **70**, 3551–3560 (1996).
125. Towers, G.J. *et al.* Cyclophilin A modulates the sensitivity of HIV-1 to host restriction factors. *Nat. Med.* **9**, 1138–1143 (2003).
126. Martin, A. & Schmid, F.X. Evolutionary stabilization of the gene-3-protein of phage fd reveals the principles that govern the thermodynamic stability of two-domain proteins. *J. Mol. Biol.* **328**, 863–875 (2003).
127. Corey, R.B. & Pauling, L. Fundamental dimensions of polypeptide chains. *Proc. R. Soc. Lond. B* **141**, 10–20 (1953).
128. Edison, A.S. Linus Pauling and the planar peptide bond. *Nat. Struct. Biol.* **8**, 201–202 (2001).
129. Christoph, C. & Wuthrich, K. NMR studies of the rates of proline cis-trans isomerization in oligopeptides. *Biopolymers* **20**, 2623–2633 (1981).
130. Kofron, J.L., Kuzmic, P., Kishore, V., Colon-Bonilla, E. & Rich, D.H. Determination of kinetic constants for peptidyl prolyl cis-trans isomerases by an improved spectrophotometric assay. *Biochemistry* **30**, 6127–6134 (1991).
131. Park, S.T., Aldape, R.A., Futer, O., DeCenzo, M.T. & Livingston, D.J. PPIase catalysis by human FK506-binding protein proceeds through a conformational twist mechanism. *J. Biol. Chem.* **267**, 3316–3324 (1992).
132. Eberhardt, E.S., Loh, S.N., Hinck, A.P. & Raines, R.T. Solvent effects on the energetics of prolyl peptide bond isomerization. *J. Am. Chem. Soc.* **114**, 5437–5439 (1992).
133. Scheiner, S. & Kern, C.W. Theoretical studies of environmental effects on protein conformation. 1. Flexibility of the peptide bond. *J. Am. Chem. Soc.* **99**, 7042–7050 (1977).
134. Kramer, M.L. & Fischer, G. FKBP-like catalysis of peptidyl-prolyl bond isomerization by micelles and membranes. *Biopolymers* **42**, 49–60 (1997).
135. Ma, L., Hsieh-Wilson, L.C. & Schultz, P.G. Antibody catalysis of peptidyl-prolyl cis-trans isomerization in the folding of RNase T1. *Proc. Natl. Acad. Sci. USA* **95**, 7251–7256 (1998).
136. Yli-Kauhaluoma, J.T. *et al.* Catalytic antibodies with peptidyl-prolyl cis-trans isomerase activity. *J. Am. Chem. Soc.* **118**, 5496–5497 (1996).
137. Fischer, S., Michnick, S. & Karplus, M. A mechanism for rotamase catalysis by the FK506 binding protein (FKBP). *Biochemistry* **32**, 13830–13837 (1993).