

# Peptidyl-prolyl *cis/trans* isomerases and transcription: is there a twist in the tail?

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Eukaryotic transcription is regulated predominantly by the post-translational modification of the participating components. One such modification is the *cis-trans* isomerization of peptidyl-prolyl bonds, which results in a conformational change in the protein involved. Enzymes that carry out this reaction include the yeast peptidyl-prolyl *cis/trans* isomerase Ess1 and its human counterpart Pin1, both of which recognize phosphorylated target motifs exclusively. Consequently, they operate together with proline-directed serine-threonine kinases and phosphatases. High-profile client proteins involved in transcription include steroid hormone receptors, cell-cycle regulators and immune mediators. Other key targets are elements of the transcription machinery, including the multiply phosphorylated carboxy-terminal domain of RNA polymerase II. Changes in isomerase activity have been shown to alter the transactivation potential, protein stability or intracellular localization of these client proteins. The resulting disruption to developmental processes and cell proliferation has been linked, in some cases, to human cancers.

Keywords: co-activators; Ess1; hormone receptors; Pin1; RNA polymerase II

EMBO reports (2007) 8, 40–45. doi:10.1038/sj.embor.7400873

## Introduction

Although the template-dependent synthesis of RNA molecules by polymerase enzymes is a linear and highly conserved process, it is subject to a plethora of regulatory inputs. The complexities of transcriptional control are not restricted to metazoans; they operate even in unicellular organisms, such as yeast. A common theme is the post-translational modification of participating components, including subunits of RNA polymerases (Sims *et al*, 2004). Prominent among these post-translational modifications is phosphorylation, which can regulate transcriptional events in several ways. A more recently identified mechanism involves conformational change, which can be regulated in a phosphorylation-dependent manner by some parvulin-like peptidyl-prolyl isomerases (PPIs). PPIs are enzymes that catalyse the *cis-trans* isomerization of peptide bonds

that are amino-terminal to proline residues in polypeptide chains (Schiene & Fischer, 2000).

The yeast parvulin-like PPI Ess1/Ptf1 (hereafter called Ess1) and its human orthologue Pin1 both consist of two domains—an N-terminal WW domain and a carboxy-terminal catalytic domain—joined by a short linker. The WW domain recognizes serine-proline (S-P) or threonine-proline (T-P) motifs, but only when the S or T is phosphorylated—that is,  $pS$ -P or  $pT$ -P (Landrieu *et al*, 2001; Lu *et al*, 2002; Verdecia *et al*, 2000). These motifs are generated by proline-directed protein kinases, such as cyclin-dependent kinases (CDKs), and mitogen-activated protein kinases (MAPKs; Chen *et al*, 2001; Loyer *et al*, 2005; Sanchez & Dynlacht, 2005). The *cis* and *trans* forms of the peptide bond between  $pS/pT$  and P exist in equilibrium; however, in proteins the *trans* form predominates and only 5–10% of peptidyl-proline bonds exist in the *cis* form (Reimer *et al*, 1998). Rotation around the bond is restricted due to its partial double-bond character and the potential effects of adjacent charged side chains, and can be rate limiting during protein folding. The catalytic domains of Ess1 and Pin1 accelerate isomerization of this bond whereby isomerization is contingent on prior phosphorylation. Although Pin1 increases the rate of isomerization considerably *in vitro*, it is not clear whether its function *in vivo* is always catalytic, rather than stoichiometric; the occupation of target sites by PPIs could result in some of the biological effects ascribed to them by out-competing the binding of other factors.

Ess1 was first identified as an essential gene in yeast, emphasizing the importance of this control mechanism (Hanes *et al*, 1989). Deletion of Ess1 in yeast, or its unique counterpart Pin1 in HeLa cells, causes cell-cycle arrest in late mitosis (Lu *et al*, 1996). However, homozygous Pin1<sup>-/-</sup> mice are viable and fertile, even though embryonal fibroblasts derived from these mice grow slowly and show cell-cycle re-entry defects (Fujimori *et al*, 1999). Furthermore, Pin1 has been shown to interact with several mitotic regulators, including Cdc25, a Cdc2-directed phosphatase, and Plk1, in a phosphorylation-dependent manner (Crenshaw *et al*, 1998; Shen *et al*, 1998). As well as these proteins, Pin1 substrates include several transcription factors for which the consequences of Pin1 action seem to be modulation of their transactivation potential, primarily through changes in protein stability and, in some cases, intracellular localization (Table 1). Furthermore, as well as constituting a select group of high-profile transcription factors involved in cell proliferation, survival and oncogenesis, known clients of Pin1 include RNA polymerase II (RNAP II)

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Submitted 11 August 2006; accepted 23 October 2006

**Table 1** | Targets, facilitating kinases and consequences of Pin1-mediated transcriptional control

Client	Motif	Kinase	Consequence	References
cJun	S63-P, S73-P	JNK/SAPKs	Transactivation	Wulf <i>et al</i> , 2001
cFos	T232-P, T325-P, T331-P, S374-P	ERKs	Transactivation	Monje <i>et al</i> , 2005
RAR $\alpha$	S77-P	Cdk7	Transactivation, degradation	Brodani <i>et al</i> , 2005
SRC-3/AIB1	ND	ND	Transactivation, degradation	Yi <i>et al</i> , 2005
c-Myc	S58-P, S62-P	ERKs	Dephosphorylation by PP2A, degradation	Yeh <i>et al</i> , 2004
$\beta$ -catenin	S246-P	ND	Stabilization, nuclear localization	Ryo <i>et al</i> , 2001
p53	S33-P, T81-P, S315-P, S46-P	p38 <sup>mapk</sup> , Cdc2, JNK/SAPKs	Stabilization, transactivation	Zacchi <i>et al</i> , 2002; Zheng <i>et al</i> , 2002; Wulf <i>et al</i> , 2002
p73	S412-P, T442-P, T482-P	p38 <sup>mapk</sup>	Stabilization, transactivation	Mantovani <i>et al</i> , 2004
p65/Rel	T254-P	ND	Stabilization, transactivation	Ryo <i>et al</i> , 2003
IRF3	S339-P	ND	Degradation	Saitoh <i>et al</i> , 2006
Rpb1	S2-P, S5-P, Cdc2/cyclin B	Cdk7, Cdk9	Dephosphorylation by Fcp1	Xu <i>et al</i> , 2003; Wilcox <i>et al</i> , 2004

Cdc2, cell division cycle 2; Cdk, cyclin-dependent kinase; ERK, extracellular signal-regulated kinase; Fcp1, TFIIF-associating CTD phosphatase-1; GSK-3 $\beta$ , glycogen synthase kinase-3 $\beta$ ; IRF3, interferon-regulatory factor 3; JNK, Jun amino-terminal kinase; ND, not yet determined; PP2A, protein phosphatase 2A; RAR $\alpha$ , retinoic-acid receptor  $\alpha$ ; SAPKs, stress-activated protein kinases; SRC-3, steroid-receptor coactivator-3.

itself, implying that Pin1 might also have a more direct, facilitating role in the mechanism of eukaryotic gene transcription.

### Controlling transactivation by activator protein 1 subunits

The N-terminal transactivation domain of cJun, which is a component of the transcription factor AP1 (activator protein 1), has been shown to require phosphorylation by Jun N-terminal kinases/stress-activated protein kinases (JNK/SAPKs) and the subsequent action of Pin1 to attain full activity (Wulf *et al*, 2001). Target genes of cJun include *cyclin D1*, and positive correlations between Pin1 and cyclin D1 expression levels in breast and oral squamous carcinomas have been observed (Miyashita *et al*, 2003; Wulf *et al*, 2001). The cJun paradigm also applies to at least one other AP1 component, as cFos interacts with Pin1 through pS/T-P motifs generated by extracellular signal-regulated kinase (ERK) phosphorylation of the C-terminal transactivation domain. Pin1 expression also augmented transcriptional responses to platelet-derived growth factor by cFos and its isolated transactivation domain (as a GAL4 fusion), although it remains unclear whether this required the isomerase activity of Pin1 (Monje *et al*, 2005). The suggestion that Pin1 is needed for cJun (and cFos) to acquire their fully active conformation has yet to be substantiated; given that both are conditional substrates for ubiquitin-dependent and ubiquitin-independent proteasomal degradation (Acquaviva *et al*, 2002), the underlying mechanism could involve changes in their stability. Other AP1 components might also require Pin1 for activation, potentially implicating it in tumour progression and metastasis.

### Stability and activity of steroid hormone receptors

The modulation of transcription factor activity through changes in stability was first exemplified by  $\beta$ -catenin and its role in Wingless/Int-related (Wnt) signalling (Logan & Nusse, 2004). Pin1 was reported to act as a positive regulator of  $\beta$ -catenin by antagonizing its interaction with the adenomatous polyposis coli protein (APC) complex, resulting in the stabilization and nuclear accumulation of  $\beta$ -catenin and the stimulation of its target genes, such as *c-myc*, *fibronectin* and *cyclin D1*, together with T-cell factor 4 (Tcf4; Ryo *et al*, 2001; Shaw, 2002). As with cJun, links between Pin1,

Wnt signalling and *cyclin D1* expression in several types of cancer have been reported (Kuramochi *et al*, 2006; Pang *et al*, 2006; Zhou & Gao, 2006).

Recently, a second mechanism has been described through which Pin1 action upregulates Tcf4/ $\beta$ -catenin in phosphatase and tensin homologue (PTEN)-negative prostate cancer cells (Chen *et al*, 2006). Despite elevated Akt-protein kinase B signalling and consequent inactivation of glycogen synthase kinase 3 $\beta$  (GSK3 $\beta$ )—the kinase presumed to be responsible for  $\beta$ -catenin phosphorylation (Ryo *et al*, 2001)—overexpression of Pin1 in these cells led to an increase in Tcf4/ $\beta$ -catenin transcriptional activity without affecting  $\beta$ -catenin levels. As the androgen receptor (AR) also uses  $\beta$ -catenin as a co-activator and might therefore compete with Tcf4 for limiting  $\beta$ -catenin, Chen and colleagues explored the effect of Pin1 on AR/ $\beta$ -catenin interactions. Pin1 was found to antagonize the interaction of  $\beta$ -catenin with the ligand-binding domain of AR, thereby preventing transactivation by the AR/ $\beta$ -catenin complex and resulting in concomitant Tcf4/ $\beta$ -catenin activation. However, it is unclear whether the mechanism involves isomerization of  $\beta$ -catenin, because the Pin1-insensitive  $\beta$ -catenin S246A mutant was not tested and the protein kinase acting in place of GSK3 $\beta$  was not identified. Indeed, significant inhibition was achieved with a catalytically inactive Pin1 mutant, which questions whether isomerization is the primary mechanism for AR/ $\beta$ -catenin downregulation.

Pin1 also encroaches on the regulation of other hormone receptor systems. Both the stabilization of and ligand-independent transcriptional activation by the retinoic-acid receptor RAR $\alpha$  require phosphorylation on S77 within its AF1 domain by Cdk7 (Kopf *et al*, 2000; Rochette-Egly *et al*, 1997), which is an atypical cyclin-dependent kinase and a component of the basal transcription factor TFIIF (Loyer *et al*, 2005). However, Pin1 overexpression destabilized RAR $\alpha$  with a consequent loss of reporter transactivation (Brodani *et al*, 2005). An S77A mutation in RAR $\alpha$  prevented its interaction with Pin1 *in vitro* and stabilized the receptor in COS cells, although at the expense of its transcriptional activity. This scenario is reminiscent of the relationship between transcriptional activation and proteasome-mediated protein degradation, which has been elegantly shown for the oestrogen receptor (Reid *et al*, 2003). In the case of RAR $\alpha$ , the role of Pin1 would be to switch the phosphorylated

receptor from the transcriptionally competent to the ubiquitination-sensitive conformation. Too much Pin1 activity would trigger premature ubiquitination of RAR $\alpha$ , thereby suppressing transcription.

The influence of Pin1 on a steroid-receptor coactivator (SRC) has also been reported. When co-expressed in HeLa cells, SRC-3, which is also known as AIB1/ACTR/pCIP/RAC3/TRAM1 (Xu & Li, 2003), and Pin1 synergistically activated progesterone receptor-mediated reporter expression (Yi *et al*, 2005). Again, Pin1 expression had a dual effect: its interaction with SRC-3 increased the amount of p300/CBP that was associated with SRC-3 and recruited to an endogenous oestrogen-responsive promoter. Pin1 also reduced the level of SRC-3 in cells in a manner that was reversible by the proteasome inhibitor MG132, again indicating that Pin1 acts as a linchpin between transcriptional activation and activator degradation.

### Conformation and stability of cell-cycle regulators

*Cis-trans* isomerization as a mechanism to control protein stability extends to several transcription factors that are prominent in cell-cycle control. In response to DNA damage, phosphorylation of p53 by stress-dependent kinases generates target motifs for Pin1, which alters the conformation of p53 such that it is less-readily bound by the E3 ubiquitin ligase Mdm2 (Zacchi *et al*, 2002; Zheng *et al*, 2002). The net result is stabilization and nuclear accumulation of p53, the activation of checkpoint controls and ultimately the upregulation of target genes, including *mdm2*, *BCL2-associated X* protein (*Bax*) and *killer/DR5*, to increase the amount of apoptosis. These responses to genotoxic insults are impaired in *Pin1*<sup>-/-</sup> mouse embryonic fibroblasts (MEFs; Berger *et al*, 2005; Wulf *et al*, 2002; Zacchi *et al*, 2002; Zheng *et al*, 2002).

Genotoxic stress also triggers apoptosis in cells lacking p53 through the action of its relatives p63 and p73 (Murray-Zmijewski *et al*, 2006). Intriguingly, Pin1 seems to be equally important in the regulation of p73. Mantovani and colleagues were able to show, in a p53-null background, that loss of Pin1 destabilized p73 under both normal and stress conditions, and compromised expression from p73 target gene promoters such as *Bax*, *p53-induced gene 3 (PIG3)* and *p53-regulated apoptosis-inducing protein 1 (p53AIP1)* (Mantovani *et al*, 2004). Treatment of cells with cisplatin or doxorubicin induced phosphorylation of p73 on Y99 by the c-Abl tyrosine kinase (Tsai & Yuan, 2003; Yuan *et al*, 1999), and subsequently on three S/T residues within the proline-rich region present in the  $\alpha$  and  $\beta$  isoforms of p73 by p38<sup>mapk</sup> (Sanchez-Prieto *et al*, 2002). The pS/T-P motifs bind Pin1, and this was enhanced by overexpression of either c-Abl or a combination of p38<sup>mapk</sup> and active MAPK kinase 6 (MKK6). Pin1 binding could be correlated with a conformational change in p73, the association of p73 with p300 and its acetylation on lysine residues. Acetylation might be important for targeting p73 to pro-apoptotic promoters (Costanzo *et al*, 2002). Overexpression of p300 also stabilized p73 in a Pin1-dependent manner, indicating that lysine acetylation might antagonize ubiquitination of p73 and its degradation by the proteasome (Mantovani *et al*, 2004).

In direct contrast to its role in promoting p53 stability, Pin1 has been shown to contribute to the ubiquitin-mediated degradation of other transcription factors, as reported for chorion factor 2 (CF2), which is a protein involved in *Drosophila* egg development (Hsu *et al*, 2001; Shaw, 2002). Another transcription factor influenced by this mechanism is the cell-cycle regulator c-Myc. In response to Ras signalling, S62 in the N terminus of c-Myc is phosphorylated by ERKs, which enhances c-Myc stability. Subsequently, as Ras signalling

subsides, GSK3 $\beta$  becomes active and phosphorylates c-Myc a second time on T58 (Sears *et al*, 2000). Phosphorylation of T58 is associated with the destabilization of c-Myc: T58 mutations have been observed in Burkitt's lymphomas, and linked to the increased stability and transforming potential of c-Myc (Dang *et al*, 2005). However, destabilization of c-Myc also requires dephosphorylation of S62 by protein phosphatase 2A (PP2A), which is a conformation-sensitive phosphatase (Zhou *et al*, 2000). It seems that Pin1 binds to c-Myc at the pS62-P and pT58-P motifs to catalyse *cis-trans* isomerization of the seryl-prolyl bond and create an ideal substrate for PP2A (Yeh *et al*, 2004). Once c-Myc is dephosphorylated at S62, it is reportedly targeted for ubiquitin-mediated degradation by the F-box protein Fbw7 (Welcker *et al*, 2004a,b; Yada *et al*, 2004); however, the recent demonstration that a second E3 ubiquitin ligase, HectH9, controls transcriptional activation by c-Myc indicates that the mechanism involves further levels of complexity (Adhikary *et al*, 2005).

### Immune mediators, conformation and stability

Nuclear factor- $\kappa$ B (NF $\kappa$ B) subunits p65/Rel and p50 accumulate in the nucleus after the cytokine-induced degradation of their cytoplasmic inhibitors, referred to as I $\kappa$ Bs (Li & Verma, 2002). Subsequent downregulation of NF $\kappa$ B is achieved, in part, by newly synthesized I $\kappa$ B, which can retrieve NF $\kappa$ B from the nucleus. However, a second mechanism seems to regulate p65/Rel. The correlation between high Pin1 expression and elevated nuclear p65 levels in breast cancer tissues prompted a study into the effect of Pin1 expression on NF $\kappa$ B activity; it was found to enhance NF $\kappa$ B DNA binding and reporter activity (Ryo *et al*, 2003). A single motif in p65/Rel (pT254-P) was recognized by Pin1. A T254A mutation in p65/Rel abolished Pin1 binding and destabilized the protein. Conversely, polyubiquitination of p65, which is mediated by the E3 ubiquitin ligase Socs1, could be inhibited by overexpression of Pin1. However, it is not clear whether isomerization by Pin1 is required for this effect or whether binding alone could be sufficient to stabilize p65/Rel. The T254A mutant of p65/Rel was also not protected by *de novo* I $\kappa$ B synthesis; however, this could have been due to its failure to activate NF $\kappa$ B-responsive genes and I $\kappa$ B expression, rather than it being refractory to I $\kappa$ B downregulation.

The interferon-regulatory factor 3 (IRF3), which is involved in gene regulation during innate immune responses, seems to have the opposite relationship with Pin1 (Saitoh *et al*, 2006). Downregulation of Pin1 expression or mutation of its conserved recognition motif (S339-P) in IRF3 prolonged double-stranded RNA (dsRNA)-induced expression from the IRF3-responsive interferon- $\beta$  (IFN- $\beta$ ) gene promoter. Conversely, Pin1 overexpression curtailed expression by accelerating the ubiquitin-dependent degradation of active IRF3 by the 26S proteasome. In line with these observations, Pin1-deficient mice overproduced IFN- $\beta$  following injection of dsRNA, and bone marrow-derived *Pin1*<sup>-/-</sup> macrophages secreted three times more IFN- $\beta$  than macrophages from *Pin1*<sup>+/+</sup> mice, which is consistent with a role for Pin1 as a negative regulator of IRF3 signalling.

The reciprocal effects of Pin1 on NF $\kappa$ B and IRF3 might have important consequences for viral immunity. Activation of type I interferon expression by inflammatory mediators and dsRNA involves the participation of NF $\kappa$ B and IRF3, respectively, in the assembly of a multiprotein complex at interferon gene promoters. Given the above findings, Pin1 might act to integrate signal input to such promoters by counterbalancing the activity of the two transcription factors within the complex. In this context, Pin1 might even affect the balance

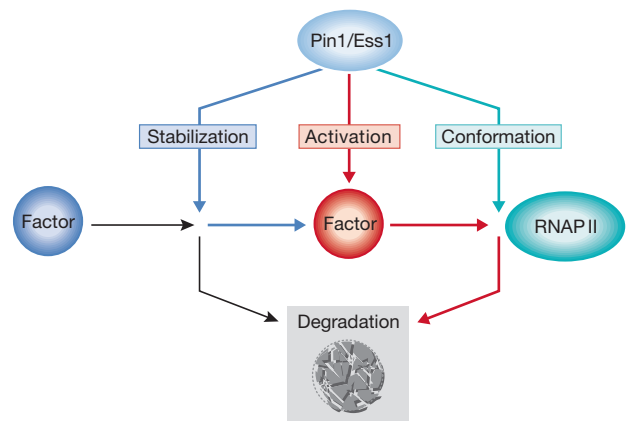
between cell death and survival, as discussed elsewhere (Goutagny *et al.*, 2006), although this provocative idea will require further work to be substantiated.

### The relationship between Pin1 and RNA polymerase II

Despite the array of high-profile transcription factors that have been identified as Pin1 client proteins, perhaps the most interesting target in the context of this review is RNAP II (Fig 1). The C-terminal domain (CTD) of the largest mammalian polymerase subunit (Rpb1) is a 50-fold repeat of the conserved heptamer Y-S2-P-T-S5-P-S. This domain is essential for cell viability and gene transcription (Allison *et al.*, 1988). The heptamer repeat is a substrate for several kinases and phosphatases. Notable among the former are the cyclin-dependent kinases Cdk7, Cdk8 and Cdk9, as well as the mitotic kinase Cdc2/cyclin B (Loyer *et al.*, 2005). Cdk7, with cyclin H, is part of the basal transcription factor TFIIF (Feaver *et al.*, 1994; Roy *et al.*, 1994) and phosphorylates S5 during transcriptional initiation. Cdk9, with cyclin T, is part of the elongation factor p-TEFb (Marshall *et al.*, 1996) and phosphorylates S2 during elongation. Cdk8, with cyclin C, is part of the Srb/mediator complex (Akoulitchev *et al.*, 2000) and, like Cdk7, phosphorylates S5 during transcriptional initiation (Liu *et al.*, 2004). By contrast, the Cdc2/cyclin B complex phosphorylates both S2 and S5, thereby inhibiting transcription (Gebara *et al.*, 1997).

At least four CTD phosphatases have been identified so far (Loyer *et al.*, 2005). TFIIF-associated CTD phosphatase1 (Fcp1) is the best characterized and is implicated in polymerase recycling (Archambault *et al.*, 1998; Cho *et al.*, 1999; Kobor *et al.*, 1999). Notably, Fcp1 is one of several multicopy suppressors identified for yeast mutants lacking *Ess1* (Pin1; Wu *et al.*, 2000). This genetic linkage of Pin1 to RNAP II has been substantiated by the demonstration of Pin1 binding to phospho-CTD fragments (Kops *et al.*, 2002; Myers *et al.*, 2001) and inhibiting CTD dephosphorylation by Fcp1 *in vitro* (Palancade *et al.*, 2004). Importantly, Pin1 has been implicated in the accumulation of the hyper-phosphorylated inactive form of RNAP II that is present in mitotic cells (Xu *et al.*, 2003). Pin1 achieves this by inhibiting CTD dephosphorylation by Fcp1, as well as by augmenting CTD phosphorylation by cdc2/cyclin B. This might be the crucial function of Pin1 in *Saccharomyces cerevisiae*, as *Ess1*-deficient yeast cells arrest in mitosis (Hanes *et al.*, 1989; Lu *et al.*, 1996). However, *Pin1*<sup>-/-</sup> MEFs complete mitosis successfully but show defects in cell cycle re-entry (Fujimori *et al.*, 1999), suggesting a second crucial role for Pin1. In *Pin1*<sup>-/-</sup> mice, this is reflected in growth and fertility defects owing to a prolonged cell cycle (rather than cell-cycle arrest or apoptosis), culminating in the complete loss of primordial germ cells from the testes and tubule degeneration by 14 months of age (Liou *et al.*, 2002; Atchison *et al.*, 2003; Atchison & Means, 2003).

Subsequent analyses have suggested a positive role for Pin1 in transcription initiation by revealing a genetic linkage between *Ess1* and several yeast CTD kinases, including Kin28 and Srb10 (orthologues of mammalian kinases Cdk7 and Cdk8). In contrast to Fcp1, which was identified as a multicopy suppressor, the defects caused by the loss of *Ess1* were improved by reducing the levels of these CTD kinases (Wilcox *et al.*, 2004). For example, phosphorylation of the CTD by Srb10, which is a subunit of the mediator complex, is thought to inhibit polymerase recycling by preventing pre-initiation complex formation (Hengartner *et al.*, 1998). Pin1 might reverse the effect of Srb10 by stimulating CTD dephosphorylation of S5 by Fcp1 (Kops *et al.*, 2002), which is consistent with the antagonistic roles of Srb10 and Fcp1. This idea is further supported by the ability



**Fig 1** | The peptidyl-prolyl isomerase Pin1/Ess1 acts in three ways in transcriptional activation. In the first scenario (blue), PPI activity protects client proteins such as  $\beta$ -catenin and p53 from proteasomal degradation, allowing them to participate in promoter binding and gene activation. In the second scenario (red), conformational change triggers ubiquitin-dependent degradation by the proteasome (grey box), which might (for example, in the case of retinoic-acid receptor  $\alpha$  or steroid-receptor coactivator-3) or might not (for example, in the case of c-Myc or interferon-regulatory factor 3) be linked to transcriptional potency. The third scenario (turquoise) involves direct interaction of Pin1 with the carboxy-terminal domain of RNA polymerase II (RNAP II), which has been implicated in downregulation of the polymerase during mitosis, polymerase recycling and elongation. PPI, peptidyl-prolyl isomerase.

of alanine substitutions at S5 in the CTD to confer viability on *ess1* mutants (Wilcox *et al.*, 2004), indicating that Pin1 activity is required for S5 dephosphorylation. An inability to turnover RNAP II rapidly could be the underlying defect that prevents the rapid expression of immediate early genes at the onset of G1, which corresponds to the phenotype of *Pin1*<sup>-/-</sup> MEFs. It would also exacerbate reductions in *cyclin D1* expression, the importance of which is highlighted by the similar phenotypes of *Pin1*-deficient and *cyclin D1*-deficient mice (Liou *et al.*, 2002).

The two scenarios described above raise an important question: is it possible that during mitosis the crucial role for Pin1 is to inhibit CTD dephosphorylation by Fcp1, whereas in G1 it has the equally important but opposite role of stimulating CTD dephosphorylation by Fcp1 to promote RNAP II recycling? In fact, Pin1 would do the same thing in both cases—that is, isomerize the *p*S-P bonds—but the consequence would only be the same if the prevailing CTD phosphorylation and conformation were the same in both situations. During mitosis, the combined activities of Cdc2/cyclin B and Pin1 might allow an Fcp1-resistant and, hence, hyper-phosphorylated CTD conformation to predominate, whereas early in G1, Pin1 activity might generate the Fcp1-sensitive CTD conformation required for RNAP II recycling. It should be noted that the relative importance of these two events might differ in yeast and mammalian cells, and that genetic interactions between *Ess1* and other CTD kinases have highlighted additional roles for Pin1 in transcription elongation (Hani *et al.*, 1999; Wilcox *et al.*, 2004), further complicating the interplay between Pin1 and the CTD of RNAP II.

**Perspectives**

Studies of Pin1 have revealed three scenarios for the involvement of peptidyl-prolyl isomerization in transcriptional regulation (Fig 1). The first relates to transcription factors that are constitutively or rapidly turned over, such as p53; Pin1 stabilizes these, allowing subsequent transactivation to occur. The second involves transcription factors for which Pin1 stimulates their degradation, as exemplified by RAR $\alpha$ ; in some cases, degradation has been tentatively linked to transactivation, hinting at a co-activator function for Pin1. The third and most complex scenario, involving the CTD of RNAP II, is one in which Pin1, in cooperation with CTD kinases and phosphatases, determines the phosphorylation and conformation of the CTD, thereby controlling the association of factors during transcription and the cell cycle.

Although the importance of parvulin-like PPIs for eukaryotic gene expression is beyond doubt, the relative importance of the numerous roles identified so far remains to be established. Presently, it is unclear why Ess1/Pin1 orthologues are essential in some but not all organisms. One explanation might be compensatory expression of other PPIs, such as cyclophilins and FK506-binding proteins (Shaw, 2002). In this regard, the existence of *Pin1*-like genes has also been proposed (Liou *et al*, 2002). However, the tiny amount of Ess1 activity necessary for growth in *S. cerevisiae* compared with the much higher levels prevailing in wild-type cells (Gemmill *et al*, 2005) challenges this explanation, instead suggesting conditional requirements for PPI activity directed towards specific targets in individual cell types, rather than a panoply of targets in every cell. Yet more complexity has been highlighted by the report of Pin1 phosphorylation on Ser16 in its WW domain (Lu *et al*, 2002), which provides scope for the regulation of Pin1 target selection during the cell cycle. In the fascinating tale of Pin1 and transcriptional regulation, there are surely more twists and turns to come.

**ACKNOWLEDGEMENTS**

Research in the author's laboratory is funded by the Biotechnology and Biological Sciences Research Council and the British Heart Foundation.

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