Landscape of Pin1 in the cell cycle

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Abstract

Pin1 is a peptidyl-prolyl isomerase which plays a critical role in many diseases including cancer and Alzheimer's disease. The essential role of Pin1 is to affect stability, localization or function of phosphoproteins by catalyzing structural changes. Among the collection of Pin1 substrates, many have been shown to be involved in regulating cell cycle progression. The cell cycle disorder caused by dysregulation of these substrates is believed to be a common phenomenon in cancer. A number of recent studies have revealed possible functions of several important Pin1-binding cell cycle regulators. Investigating the involvement of Pin1 in the cell cycle may assist in the development of future cancer therapeutics. In this review, we summarize current knowledge regarding the network of Pin1 substrates and Pin1 regulators in cell cycle progression. In G1/S progression, cyclin D1, RB, p53, p27, and cyclin E are all well-known cell cycle regulators that are modulated by Pin1. During G2/M transition, our lab has shown that Aurora A suppresses Pin1 activity through phosphorylation at Ser16 and cooperates with hBora to modulate G2/M transition. We conclude that Pin1 may be thought of as a molecular timer which modulates cell cycle progression networks.

Keywords: Pin1, cell cycle, Aurora A, hBora

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Introduction

Pin1 (peptidyl-prolyl cis-trans isomerase) is a unique enzyme that specifically isomerizes phospho-Ser-Pro or phospho-Thr-Pro (pSer/Thr-Pro) peptide bonds, and its dysregulation has been implicated in a number of pathological conditions, including aging, immune response, apoptosis, Alzheimer's disease and multiple cancers.^{1–5} Up-regulation of Pin1 has been observed in many types of cancers including lung, breast, esophageal squamous cell carcinoma (ESCC) and prostate cancers, and is generally regarded as a biomarker for poor prognosis.^{6,7} Several studies have reported possible mechanisms of Pin1 up-regulation. For example, increased Pin1 level was found to be increased through the oncogenic Neu/Ras pathway via E2F to promote tumorigenesis in mammary epithelial cells.⁸ In addition, Pin1 interacts with Notch1 and Notch4 to activate their transcriptional activity. The activated Notch1 and Notch4 directly induce transcription of Pin1 to form positive feedback loop.^{9,10} In prostate cancer, dramatic decreases in miR-296-5p resulted in increased levels of Pin1,¹¹ miR200c down-regulate Pin1 expression both in mRNA and protein to decrease the abundance and self-renewal activity of breast cancer stem cells.¹² These results

demonstrate an alternative regulatory mechanism of Pin1 and emphasize the importance of Pin1. Because many Pin1 substrates are critical cell cycle regulators (such as cyclin D1 and RB), up-regulation of Pin1 is thought to promote tumor progression.

Pin1 contains an N-terminal WW domain and a C-terminal PPlase domain. Client proteins containing the pSer/Thr-Pro motif are recognized by the WW domain and isomerization of the peptide bond is catalyzed by the PPlase domain. Pin1-mediated protein isomerization is correlated to several functional outcomes for client proteins, including alterations in catalytic activity, protein stability, interactions with other proteins, and subcellular localization.^{5,13} Phosphorylation is the most common post-translational modification in cells, and global mass spectrometry analysis has revealed that over 96% of all phospho-proteins in the cell are phosphorylated at serine and threonine residues.¹⁴ Because Ser/Thr phosphorylation is a widespread and crucial mechanism of signal transduction, tight regulation of phospho-protein function becomes an important issue. Thus, Pin-mediated isomerization of pSer/Thr-Pro motif containing proteins represents a critical mechanism in signal transduction.

Table 1 Pin1 substrates and regulators in cell cycle

Pin1 substrates			
Protein	Pin1 function	Pin1 binding motif	References
G1/S phase			
cyclin D1	Stabilization, localization, transcription	Thr286	22,26,27
RB	Inactivation	Ser608 and Ser612	28
p53	Stabilization and activation	Ser33, Ser46, Thr81 and Ser315	23,29–36
p27	Stabilization	Thr187	24
cyclin E	Degradation	Thr380	39
G2/M phase			
hBora	Degradation	Ser274 and Ser278	21
CDC25	Inhibit phosphatase activity	Thr48 and Thr67	16
Wee1	Inactivation	Thr186	47
Pin1 regulators			
Protein	Function	Pin1 outcome	References
E2F	Transcriptional activation	Increase protein level	8
Notch1	Transcriptional activation	Increase protein level	9,10
Notch4	Transcriptional activation	Increase protein level	9,10
DAPK1	phosphorylation at Ser71	Inhibit catalytic activity	41
PLK1	phosphorylation at Ser56	Stabilization	16,46
Aurora A	phosphorylation at Ser16	Inhibit binding ability	21
PKA	phosphorylation at Ser16	Inhibit binding ability	18
PP2A	Dephosphorylation at Ser16	Recover binding ability	44
miR-296-5p	Translational repression	Decrease protein level	11
miR-200c	mRNA degradation	Decrease protein level	12

Pin1 regulation of the cell cycle

In normal cell physiology, Cyclin/Cdk complexes regulate progression through the cell cycle, and their directed degradation is often responsible for triggering advancement through the phases. The cell cycle is a phosphorylation dependent progression and many of the phosphorylated proteins are regulated by Pin1 (Table 1). In addition, many kinases that act on Ser/Thr-Pro motifs, such as cyclin-dependent kinases (CDKs), Jun-N-terminal protein kinases (JNKs), polo-like kinases (PLKs) and glycogen synthase kinases 3 (GSK3) play major roles in cell cycle regulation. Accordingly, Pin1 may contribute to cell cycle regulation through isomerization of client proteins which are phosphorylated by CDKs, JNKs, PLKs and GSK3. Furthermore, Pin1 was first identified as a mitosis regulator and subsequent studies have solidified and further elucidated the importance of Pin1 in G2-M phase.^{15,16} Moreover, recent studies have revealed an additional role for Pin1 in controlling G1/S phase.^{8,17,18} Several studies have also shown that decreased Pin1 expression leads to cell cycle attenuation.^{15,19–21} Overall, Pin1 plays a critical role as a cell cycle modulator to promote cell cycle progression. However, the mechanisms by which Pin1 is regulated remain unclear. Pin1 protein level does not significantly fluctuate during cell cycle progression,^{19–21} but the binding partners of Pin1 are dynamically regulated by transcriptional and post-transcriptional mechanisms. Many Pin1

partners are critical cell cycle regulators that are expressed or activated in a specific cell cycle phase to tightly regulate cell cycle progression (ex. Cyclin D1 only exists in G1 phase and hBora accumulates in G2 phase). Therefore, Pin1 may regulate specific substrates as they appear in different phases of cell cycle. Discovering mechanisms to regulate the activity of Pin1 has become an important pursuit in understanding control of cell cycle progression. Recently, we found that Pin1 may be regulated either by miRNA or post-translational modification by Aurora A (Table 1).^{11,21} In the following sections, we will describe specific molecular interactions that taken together comprise the landscape of Pin1 activity in cell cycle progression (Figure 1).

Pin1 in G1/S phase

The role of Pin1 in G1/S phase is relatively unknown compared to G2/M phase. However, several known Pin1 substrates, such as cyclin D1, RB, p53 and p27, are centrally involved in G1/S phase.^{17,22–24} In addition, E2F, which is a G1-S transition initiator, is known to enhance Pin1 expression in G1/S phase.⁸ All the partners of Pin1 described in this review are critical regulators of the cell cycle and have tight connectivity in cell cycle progression. This is clearly true for the Pin1 interacting proteins that are involved in G1-S phase. During early G1 phase, E2F is bound and inhibited by RB, whereas the interaction is disrupted by RB phosphorylation in mid-G1 phase. Once E2F is released from RB,

Figure 1 Pin1 regulating network in cell cycle. During G1/S phase, Pin1 promotes cyclin D1 overexpression and RB phosphorylation. The phosphorylated RB releases E2F, which is a transcriptional factor to enhance Pin1 expression. Pin1 stimulates cyclin E degradation in S phase. During G2 phase, Aurora A inactivates Pin1 and active PLK1 result in CDC25 activation triggers G2/M transition. During the G2/M transition, GSK-3 β interacts with and phosphorylates hBora at S274/S278, and in the meantime, Aurora A interacts with and phosphorylates Pin1 at Ser16 to disrupt Pin1 function by suppressing Pin1 binding to phospho-hBora and thus preventing β -TrCP-mediated premature hBora degradation. Therefore, Aurora A forms a complex with phospho-hBora to phosphorylate Plk1 at Thr210 and activates Plk1. Plk1 activates CDC25 result in the activation of the Cyclin-B1/CDK1 complex and promotes mitotic entry. After mitotic entry, Pin1 could recover the binding activity and enhance protein stability through PP2A and PLK1. The active Pin1 promotes the degradation of hBora. Aurora A is then available to bind with TPX2 result in mitotic spindle assembly. Wee1 is phosphorylated at Tyr168 by CDK1 and Pin1 isomerize phosphorylated Wee1 inactivation.

it will turn on cyclin D1 transcription to trigger cell cycle progression in G1 phase.²⁵ In this section, we will provide an overview of the role of Pin1 in G1/S phase.

Pin1 and cyclin D1

Cyclin D1 is an essential factor for cell cycle progression, especially in G0, G1 and S phase. Pin1 increases cyclin D1 expression through transcriptional and post-translational regulation. Up-regulation of Pin1 has been demonstrated to increase cyclin D1 mRNA expression through c-Jun/AP-1 and β -catenin/TCF mediated transactivation.^{26,27} In addition, Pin1 directly interacts with cyclin D1 at the phosphorylated Thr286-Pro motif and catalyzes isomerization of cyclin D1. This interaction between Pin1 and cyclin D1 inhibits nuclear export of cyclin D1, resulting in stabilization and accumulation of cyclin D1 in the nucleus.²²

Pin1 and RB

RB, which is a key regulator of G1-S transition, interacts with E2F and inhibits E2F activity in early G1 phase, until RB is phosphorylated by CDKs.²⁵ Pin1 directly binds to pRB and triggers RB hyperphosphorylation, resulting in E2F release and activation.²⁸ Subsequently, activation of E2F initiates G1-S phase transition. Moreover, activated E2F can bind to the Pin1 promoter and enhance Pin1 expression to form a positive feedback loop.

Pin1 and p53

The activation of p53 is a well-known response to DNA damage, which causes cell cycle arrest or apoptosis. Under normal conditions, MDM2 binds to p53 and

promotes ubiquitin-mediated proteasome degradation. DNA damage specifically induces p53 phosphorylation on Ser/Thr-Pro motifs.²³ There are four Ser-Pro motifs (Ser33, Ser46, Ser127, and Ser315) and two Thr-Pro motifs (Thr81 and Thr150) in p53. DNA damage induces Pin1 phosphorylation on Ser46 by HIPK2, PKC δ and P38, while phosphorylation of Thr81 is induced by JNK.^{29–32} Pin1 binds to Thr81 phosphorylated p53 and prevents the interaction between p53 and MDM2, thereby promoting p53 stabilization.^{33,34} In addition, Pin1 is required for p53 transcriptional activity. Pin1 is recruited to p53-responsive promoters to promote binding and acetylation by p300 after DNA damage. Furthermore, when p53 is phosphorylated at Ser46, Pin1 promotes dissociation of p53 from the apoptosis inhibitor iASPP.³⁵ Interestingly, Pin1 also binds and stabilizes HIPK2, which is known to phosphorylate p53 on Ser46. Therefore, this interaction forms a feedback loop by enhancing phosphorylation of p53 at Ser46 to block interaction of iASPP and promote apoptosis.^{35,36}

Pin1 and p27

p27/Kip inhibits checkpoint kinase CDK2/cyclinE and functions as a negative regulator of G1-S transition. The activity of p27 is regulated by phosphorylation, synthesis and degradation. CDK2 phosphorylates p27 at Thr187, and this phosphorylation allows association between p27 and the SCF complex, triggering the proteolytic degradation of p27. The degradation of p27 prevents cell cycle arrest at G1 phase.³⁷ It has been shown that the phosphorylated Thr-187-Pro motif in p27 is a functional Pin1 binding site. Therefore, Pin1 catalyzes a conformational change in p27, and thus mediates p27 stability and G1-S transition.²⁴

Pin1 and cyclin E

Cyclin E is synthesized and interacts with CDK2 during G1-S transition. This complex phosphorylates RB1 and then in turn releases and activates E2F downstream genes required to execute the G1-S transition. Therefore, cyclin E degradation is required for proper cell cycle progression. GSK3 β phosphorylates cyclin E at the Thr380-Pro motif.³⁸ Phosphorylated cyclin E interacts with Pin1,³⁹ and the Pin1-cyclin E complex then interacts with E3 ligase (FBXW7a). This interaction triggers Pin1 to catalyze a conformational change in cyclin E, leading to further interaction with another E3 ligase (FBXW7 γ), which results in the degradation of cyclin E.² Accordingly, Pin1 mediates cyclin E degradation in G1-S transition.

Pin1 in centrosome amplification

Pin1 is involved in the regulation of centrosome duplication, and overexpression of Pin1 interferes with proper centrosome amplification, resulting in multipolar spindles in mitosis, chromosome missegregation and aneuploidy.⁴⁰ This effect can be modulated by Death-associated protein kinase 1 (DAPK1). DAPK1 is a positive mediator of gamma-interferon induced programmed cell death, and inactivates Pin1 PPIase domain by phosphorylation on Ser71. It was previously shown that DAPK1 can suppress Pin1-mediated centrosome amplification and cell transformation.⁴¹

Pin1 in G2/M phase

Pin1 and the hBora/Aurora A network

The function of Pin1 was first revealed as a negative regulator in mitosis. Evidence for this came from modulating Pin1 protein levels in HeLa cells. Pin1 depletion was found to cause premature entry into mitosis and mitotic arrest, whereas the overexpression of Pin1 inhibited the G2/M transition.^{15,16,19} These results suggested that Pin1 is essential for mitotic regulation and acts as a negative regulator of the G2/M transition by attenuating mitosis-promoting factor activity. However, the mechanism of this negative regulation is still unclear. Recently, our lab has described a cooperation between the regulatory network of Pin1 and Aurora A/hBora to modulate G2/M transition.^{21,42} Since Pin1 levels do not significantly fluctuate during cell-cycle progression,¹⁹ post-translational modification is likely a critical regulatory mechanism to alter Pin1 function. Several kinases and phosphatases have been shown to activate or stabilize Pin1 (Table 1). These include Aurora A and PKA, both of which phosphorylate Pin1 at Ser16 and inhibit Pin1 activity.

hBora, which is an activator of Aurora A, is phosphorylated by glycogen synthase kinase 3 β (GSK3 β) at Ser274 and Ser278, resulting in hBora accumulation in G2 phase. Phosphorylated-hBora cooperates with Aurora A to phosphorylate and activate PLK1 at Thr210 and promote mitotic entry.⁴³ Activated PLK1, in turn, activates CDC25, which leads to the dephosphorylation of Thr14 and Tyr15 in CDK1, and triggers activation of the Cyclin-B/CDK1 complex. This activation initiates mitotic entry. Thus, the GSK3 β -mediated phosphorylation of hBora in G2 phase is

an essential event for mitotic entry. In our studies, we found that Pin1 promotes hBora degradation through the β -TrCP-mediated ubiquitin-proteasome pathway. Furthermore, Pin1 blocks CDC25 catalytic activity to attenuate G2/M transition. During G2, Aurora A phosphorylates Pin1 to block its substrate binding ability and induces translocation into cytosol.²¹ Taken together, Aurora A abolishes Pin1 mediated hBora degradation and CDC25 inhibition in G2 phase to allow mitotic entry.

Oh and Malter reported that Pin1 Ser16 is up-regulated under treatment of calyculin A, which is PP2A inhibitor.⁴⁴ The result showed that PP2A dephosphorylated Pin1 at Ser16 and recover protein binding ability of Pin1. Besides, PP2A activation is important to G2/M checkpoint. Inactive PP2A decrease CDK activity and delay mitotic entry.⁴⁵ Taken together, after mitotic entry, PP2A might dephosphorylates Pin1 at Ser16 to recover binding ability, and PLK1 phosphorylates Pin1 at Ser56 to increase stability. Taken together, after mitotic entry, PP2A might dephosphorylates Pin1 at Ser16 to recover binding ability,⁴⁴ and PLK1 phosphorylates Pin1 at Ser56 to increase Pin1 stability.⁴⁶

GSK3 β -mediated phosphorylation of hBora at pSer274 and pSer278 creates potential Pin1 binding sites. One of our previous studies revealed that Ser274 and Ser278 double phosphorylated hBora was recognized by Pin1, and Pin1-mediated isomerization led to β -TrCP mediated proteasomal degradation. Degradation of hBora could release Aurora A and encourage the formation of an Aurora A-TPX2 complex. This complex targets Aurora A to the centrosomes and spindle poles and, thus, redirects Aurora A kinase activity toward distinct substrates to promote mitotic progression.⁴³ In summary, we have described the role of Pin1 control of mitotic entry and exit. Aurora A-mediated Pin1-Ser16 phosphorylation disrupts the substrate-binding ability of Pin1 to prevent phospho-hBora from premature β -TrCP-mediated degradation at the G2/M transition. However, the activity of Pin1 is recovered through dephosphorylation at Ser16 by PP2A in mitosis. Moreover, PLK1 phosphorylation of Pin1 at Ser65 may enhance Pin1 stability by inhibiting its ubiquitination without altering its isomerase activity.⁴⁶ During mitosis, active and stabilized Pin1 promotes degradation of hBora and triggers mitotic exit.

Pin1 and Wee1

Wee1 phosphorylates CDK1 on Tyr15 and inhibits its kinase activity, leading to prevention of mitotic entry in eukaryotic cells. During mitotic entry and M phase, Wee1 must be down-regulated to allow activation of CDK1 by CDC25 phosphatase. In M phase, phosphorylation of Wee1 at Ser53 and Ser124 by PLK1 and CDK1, respectively, down-regulates Wee1 by β -TrCP mediated proteasome degradation. In addition to degradation, another Pin1-dependent mechanism was reported to inactivate Wee1 in M phase. During mitotic entry, CDK1 phosphorylates Wee1 at Ser168 to form a Pin1 binding motif. Pin1 then isomerizes phosphorylated Wee1 and blocks its activity to promote cell cycle progression.⁴⁷

Conclusion

Pin1 acts as an important post-translational regulator for signal transduction and provides a molecular timer to regulate the function of many phosphoproteins, by altering protein-protein interactions, substrate dephosphorylation and degradation.^{2,5} Over the last decade, more than 40 proteins have been identified as Pin1 targets. Most of these are involved in cell-cycle regulation, cell growth and proliferation. However, little is known about the mechanism by which Pin1 is precisely 'turned on' and 'turned off' to promote cell cycle progression. Recently, we described the biological role of Pin1 Ser16 phosphorylation to address the unsolved question of how Pin1 acts as a negative regulator at the G2/M transition.¹⁵ However, the mechanism by which Pin1 modulates G1/S progression is awaiting discovery. Cell cycle dysregulation is highly correlated with several diseases, especially cancers. While Pin1 has been reported to be a tumor suppressor,⁴⁸ other data demonstrate that Pin1 is overexpressed in several cancers, suggesting that it may act as a promoter.² Clarification of the mechanisms by which the switch between active and inactive forms of Pin1 occurs in the cell cycle may help to define the role of Pin1 in cancer.

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