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Review

Pin1: Intimate involvement with the regulatory protein kinase networks in the global phosphorylation landscape☆,☆☆

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ABSTRACT

Background: Protein phosphorylation is a universal regulatory mechanism that involves an extensive network of protein kinases. The discovery of the phosphorylation-dependent peptidyl-prolyl isomerase Pin1 added an additional layer of complexity to these regulatory networks.
Scope of review: We have evaluated interactions between Pin1 and the regulatory kinome and proline-dependent phosphoproteome taking into consideration findings from targeted studies as well as data that has emerged from systematic phosphoproteomic workflows and from curated protein interaction databases.
Major conclusions: The relationship between Pin1 and the regulatory protein kinase networks is not restricted simply to the recognition of proteins that are substrates for proline-directed kinases. In this respect, Pin1 itself is phosphorylated in cells by protein kinases that modulate its functional properties. Furthermore, the phosphorylation-dependent targets of Pin1 include a number of protein kinases as well as other enzymes such as phosphatases and regulatory subunits of kinases that modulate the actions of protein kinases.
General significance: As a result of its interactions with numerous protein kinases and their substrates, as well as itself being a target for phosphorylation, Pin1 has an intricate relationship with the regulatory protein kinase and phosphoproteomic networks that orchestrate complex cellular processes and respond to environmental cues.
This article is part of a Special Issue entitled Proline-directed Foldases: Cell Signaling Catalysts and Drug Targets.

1. Introduction

When reversible protein phosphorylation was originally revealed as a regulatory mechanism [1,2], the extent of its impact on biological processes could not have been imagined. In the decades following the discovery of the phosphorylation of glycogen phosphorylase, and the characterization of phosphorylase kinase, other phosphorylated proteins and protein kinases have been identified at an accelerating pace [3–5]. The exponential increase in the number of identified phosphorylation sites and protein kinases coincided with predictions that approximately one-third of cellular proteins would be phosphorylated by as many as "A Thousand and One Protein Kinases" in humans [6,7]. While the total number of protein kinases was an over-estimate, largely because the human genome revealed several-fold less genes than initially anticipated, the overall percentage of genes that encode protein kinases was perhaps unexpectedly high [8,9]. In this respect, based on sequence relationships, the genome of the budding yeast *Saccharomyces cerevisiae* encodes >100 protein kinases, representing approximately 2% of the genome [8]. This percentage has proven to be similar in other organisms including humans where >500 genes encoding protein kinases have been identified [9]. It is also evident that the proportion of proteins that are phosphorylated at some stage during their life cycle is greater than initially estimated. In fact, a recent "ultradeep" phosphoproteomics study identified phosphorylated residues on more than 75% of the cellular proteins detected [10]. In that study, more than 50,000 phosphopeptides (representing >38,000 identified phosphorylation sites) were identified from approximately 11,000 proteins from a single cell line indicating that each of the target proteins was phosphorylated at several distinct sites (>3 sites/protein) [10]. These data demonstrate the extent and depth to which protein phosphorylation is distributed throughout the proteome. On another level, just as the genome projects represented the end of the discovery phase for the identification of new protein kinases (with the possible exception of atypical protein kinases), ultradeep phosphoproteomics signals the end of the discovery phase for the identification of phosphorylation sites.

☆ Note: A list of abbreviations for protein names is available as Supplementary Table 1.
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2. Beyond the global phosphorylation landscape

The explosion in our understanding, or at least the extensive cataloging of phosphorylated residues, has coincided with the recognition that while protein phosphorylation is widespread, protein kinases rarely act unilaterally to regulate cellular processes [11–13]. The notion that cellular events are regulated exclusively by protein kinases arranged in linear pathways has given way to the recognition that protein kinases are organized in intricate and dynamic networks that orchestrate cellular responses to cues from the environment or related to the intracellular state of the cells [14]. Elucidating the complex regulatory modifications for certain proteins as exemplified by histones in the Histone Code [15,16] and by p53 in its role as guardian of the genome [17,18], have revealed how individual modifications can impact additional modifications, and how the combined actions of these modifications contribute to the precise regulation of intricate cellular processes. Large-scale proteomics studies have demonstrated that phosphorylation is not the only widespread modification and that histones and p53 are by no means the only proteins subject to complex arrays of post-translational modifications [19–21]. At the level of individual substrate proteins, the actions of protein kinases are integrated with other regulatory enzymes including phosphatases that oppose the actions of kinases [22,23], proteases that cleave substrate proteins for processing or destruction [24–26] and a plethora of other enzymes that catalyze the addition or removal of other post-translational modifications [19].

3. Pin1: a phosphorylation-dependent peptidyl–prolyl isomerase

Another major modification of cellular proteins occurs when they are acted upon by peptidyl–prolyl isomerases that catalyze the cis–trans isomerization of peptidyl–prolyl bonds [27,28]. Although peptidyl–prolyl isomerization was originally considered to be important primarily for proper folding of proteins, there were suggestions that peptidyl–prolyl isomerization could have regulatory functions [29]. The members of the peptidyl–prolyl isomerase family also attracted attention when they were identified as the targets of immunosuppressive drugs such as cyclosporin and FK506 that target members of the cyclophilin and FKBP families of peptidyl–prolyl isomerases, respectively [30–32]. The discovery of Pin1, a member of the parvulin family of peptidyl–prolyl isomerases, added another dimension to the cellular roles of peptidyl–prolyl isomerases [33]. Pin1 was identified as an interaction partner of the NIMA protein kinase using a yeast two-hybrid screen and demonstrated to be essential for mitotic progression in human cells [33]. Pin1 was also shown to functionally complement the previously identified *ESS1* gene of *S. cerevisiae* demonstrating evolutionary conservation of function [33,34]. Notably, *ESS1* is the only peptidyl–prolyl isomerase that is essential for viability in budding yeast suggesting that Pin1 and its homologs could have indispensible regulatory functions [35,36].

Pin1 is a relatively small protein (163 amino acids in humans) comprised of two functional domains, an N-terminal WW domain that precedes its peptidyl–prolyl isomerase domain [37]. The high resolution structure of Pin1 unexpectedly revealed the prospect of preferential recognition of phosphorylated motifs for its peptidyl–prolyl isomerase activity [37], an intriguing finding given the acute activation of proline-directed protein kinases such as CDK1 and the accompanying burst of phosphorylation of numerous Ser-Pro or Thr-Pro motifs in mitotic cells [38–42]. Subsequent studies revealed that the N-terminal WW domain of Pin1 also exhibits a preference for the recognition of phosphorylated Ser-Pro or Thr-Pro motifs similar to those recognized by its isomerase domain [43,44]. Collectively, these observations were consistent with the prediction that Pin1 promotes mitotic progression by catalyzing conformational changes of mitotic phosphoproteins [45,46].

4. Regulation of mitosis by Pin1 reveals relationships with multiple protein kinases and phosphoproteins

Based on observations described above, it was appealing to hypothesize that Pin1 contributes to the dramatic architectural changes that occur as cells progress through mitosis by catalyzing conformational changes in proteins phosphorylated by mitotic proline-directed kinases [44–46]. Indeed, Pin1 was subsequently shown to exhibit phosphorylation-dependent interactions with a diverse repertoire of mitotic phosphoproteins including protein kinases such as Myt1, Wee1, Plk1, and CK2 as well as the Aurora A kinase/Bora complex that are involved in mitotic regulation [47–53]. These results implied that Pin1 could exert actions by interacting both with the regulatory protein kinase network and with substrates of these kinases (Figs. 1 and 2). Whereas it was abundantly clear that Pin1 had extensive interactions with the mitotic phosphoproteome [44,52], there was less evidence

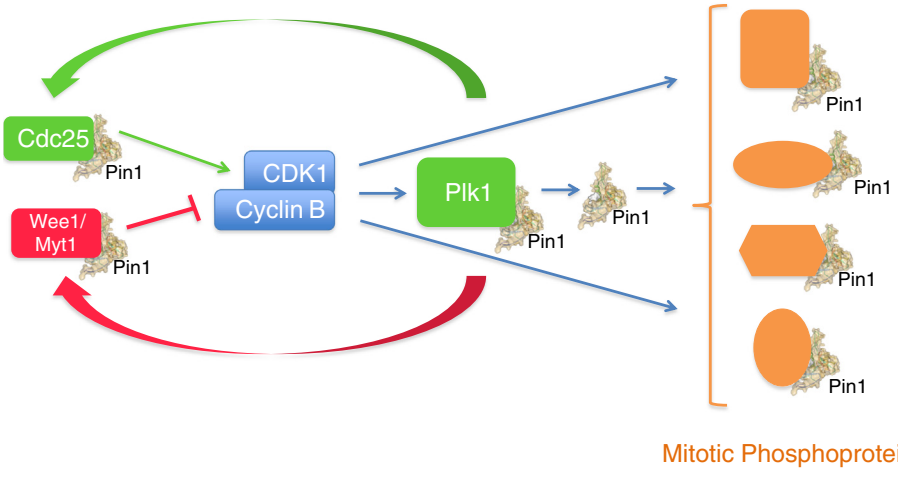

Fig. 1. Interactions of Pin1 within a regulatory circuit that controls mitotic entry. The CDK1/cyclin B complex is a critical regulator of entry into mitosis. Activation of this complex requires the actions of Cdc25C that dephosphorylates negative regulatory sites that are phosphorylated by Wee1 and/or Myt1. Dephosphorylation of CDK1/cyclin B promotes activation of Plk-1 and feedback loops that further activate Cdc25C and inactivate Wee1/Myt1. Plk1 also phosphorylates Pin1 to inhibit its ubiquitination and prevent its degradation. Pin1 interacts with several members of this regulatory circuit as well as many mitotic phosphoproteins that are phosphorylated by CDK1/cyclin B, Plk1 and/or other mitotic protein kinases. This regulatory circuit reveals the intricate role(s) of Pin1 in the precise orchestration of complex cellular processes. See text and Table 1 for more explanation and citations.

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