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REVIEW ARTICLE

Cellular Dynamics Controlled by Phosphatases

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Abstract | Protein phosphorylation, a fundamental post-translation modification that acts as a backbone of signaling networks, is essential for multiple aspects of eukaryote physiology. Phosphorylation status of a substrate is dependent on opposing activities of two distinct enzymes, where the relevant kinase catalyzes the modification and is reversed by a phosphatase. Historically, kinases have been at the research forefront; however, phosphatases have gained importance with many studies revealing predominant roles for these enzymes in controlling the cellular responses. Phosphatases are known to attenuate or amplify signaling by operating both as early, as well as delayed regulators of signal transduction. This review is focused on describing the versatile roles of phosphatases in controlling different cellular pathways through their spatio-temporal dynamics during signaling.

Keywords: *Phosphatases, Signaling, PPP family, PPM, MAPK, Mitosis*

Post-translation modification: Post-translational modification is covalent modification of proteins that occur during or after protein synthesis, where functional groups are added by enzymatic action.

Phosphatase: Enzymes that remove phosphate group from substrates by hydrolysis.

1 Introduction

Phosphorylation is a critical reversible post-translation modification known to function in various basic cellular processes, including cell growth, metabolism, cell cycle, migration, differentiation, vesicular trafficking, immunity, memory, and learning.[1–3] Protein kinases transfer phosphate group from ATP to target proteins, specifically at serine, threonine, and tyrosine residues, whereas phosphatases remove this chemical group from substrates. Protein kinases, one of largest families of genes, are extensively studied and many kinases are popular drug targets in pharmaceutical industries. But unlike their counterpart kinases, phosphatases have stayed away from limelight and were mostly considered as housekeeping enzymes. However, in recent years, several studies had made it eminently clear that phosphatases are equally crucial in regulation of cellular processes.[4] Although phosphatases were considered as simply 'switch offs' to prevent signaling overdrive, kinases are inactivated even in the presence of continuing stimuli, which suggests that phosphatases are critical in regulating the duration and strength of kinase activation. From a biochemical perspective, the enzymatic potential of a phosphatase may be higher to that of a respective kinase, owing to the fact that kinases require ATP whereas dephosphorylation is direct. Thus, it is conceivable that controlled signal transduction does not occur through kinase activation, but rather by the regulation of phosphatase expression and activity. In support of this, phosphatases, rather than the kinases looked to be under much more dynamic regulation, both at their expression as well as activity levels. Overall, kinases were believed to control the amplitude whereas phosphatases control rate and duration of signaling response.[5, 6] Thus, any imbalance in the activities of kinases and phosphatases may result in various human malignancies.

2 Diverse Family of Phosphatases

Originally phosphatases were classified into three distinct categories, Ser/Thr phosphatases, Tyr Phosphatases, and dual-specificity phosphatases, based on their substrate specificity. However, several examples have emerged over time where phosphatases were shown to dephosphorylate more than one type of substrate. Thus, a newer and more acceptable classification has been evolved, where phosphatases have been divided

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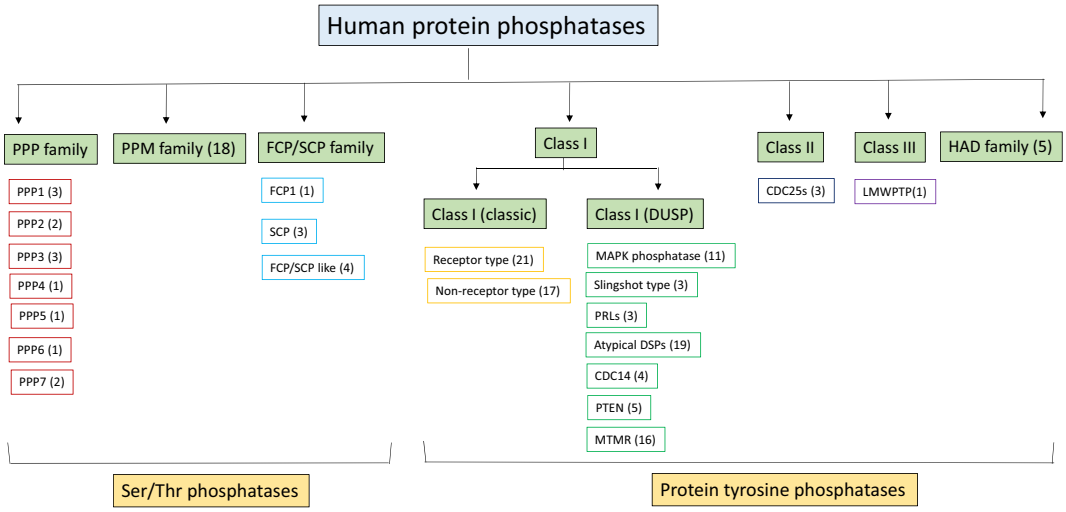

Figure 1: Human protein phosphatases classification. Classification of protein phosphatases based on their substrate specificity and sequence similarity. Numbers in the brackets indicates number of genes encoding protein phosphatases in that group. Only catalytic subunits of protein phosphatases are included. ***PPP*** protein phosphatase P, ***PPM*** protein phosphatase M sequence family, ***MAPK phosphatase*** MAP kinase phosphatases, ***PRLs*** phosphatase of regenerating liver, ***Atypical DSPs*** atypical dual-specificity phosphatases, ***MTMR*** myotubularin phosphatases, ***LMW-PTP*** low-molecular-weight protein tyrosine phosphatases, ***FCP/SCP*** TFIIF-associating component of RNA polymerase II CTD phosphatase/small CTD phosphatase, ***HAD*** haloacid dehalogenase; sequence family.

into distinct families based on sequence conservation, catalytic mechanism, and structure (Fig. 1). It is important to note that this newer classification overlaps, but does not coincide with older classification made based on substrate specificity.

The Ser/Thr phosphatases constitute three structurally distinct families (1) PPM family, (2) PPP family, and (3) the Asp-based FCP/SCP family. PPM family members, containing a PP2C catalytic domain, function as phosphatase dependent on divalent cations such as magnesium and manganese (Mg^{2+}/Mn^{2+}).[7] In contrast to PPP phosphatases, PPM family phosphatases function as monomers and have specific domains at N or C-terminus that function in substrate selection and cellular localization. In humans, at least 18 members of PPM family are known and 7 in yeast whereas in *A. thaliana* 72 members are known.[8] Unlike PPP family, PPM phosphatases are resistant to inhibitors such as okadaic acid, calyculin A, and microcystin. PPM family of phosphatases plays critical role in regulating the stress response, cell cycle progression, apoptosis, Ca^{2+} signaling, metabolism, RNA splicing, mitochondrial function, and lipid transfer. Several members of this phosphatase family have been attributed to function either as tumor suppressors or oncogenes. For example, PPM1A, PPM1B, ILKAP, and PHLPP have been shown to function as tumor suppressors whereas PPM1D is a known oncogene.

Holoenzymes: Enzymes that contain multiple protein subunits

PPP family that contains PP1-PP7 catalytic subunits catalyze majority of dephosphorylation reactions in eukaryotic cells. All the members of PPP family phosphatases have similar core structure and catalytic mechanism but have distinct set of substrates and interacting proteins. One of the important aspects of PPP phosphatases is their ability to function as holoenzymes. Numerous distinct regulatory subunits associate with PPP catalytic subunits to form heterodimeric or trimeric phosphatase holoenzymes with unique substrate specificities, subcellular locations, and physiological functions. For example, PP2A phosphatase forms heterotrimeric complex made up of ABC subunits. A subunit act as a scaffold whereas the B subunits act as regulatory proteins.[9, 10] The heterotrimeric PP2A holoenzymes display exquisite substrate specificity dependent on its regulatory subunits. For instance, B′ subunit (PPP2R5), but not B (PPP2R2) subunit, containing PP2A can interact with a centromeric protein Shugoshin whereas the B (PPP2R2) subunit, but not B′ subunit (PPP2R5), complexed PP2A specifically dephosphorylates microtubule-binding protein Tau.

In contrast to PPP and PPM phosphatases, members of FCP/SCP family depend on the

aspartic acid of DxDxT/V motif for their phosphatase activity. Another distinct feature of FCP/SCP phosphatases is that they have only one primary substrate, CTD of RNA Pol II.[11] It is well known that Pol II binds to pre-initiation complex with a hypo-phosphorylated CTD and then get phosphorylated upon binding. During transcription elongation, Ser-2 and Ser-5 get phosphorylated in heptapeptide repeat region and CTD is dephosphorylated at termination which is important for binding to new initiation complex.[12] Thus, FCP phosphatases by dephosphorylating these residues are critical during transcription where they spatially and temporally control the phosphorylation status of RNA pol II.

Protein tyrosine phosphatases (PTPs) characterized by the presence of conserved catalytic motif CX5R are divided into four distinct families based upon their structure and sequence (1) Class I Cys-based PTPs, (2) Class II Cys-based PTPs, (3) Class III Cys-based PTPs, and (4) Asp-based HAD family. The class I PTPs are further divided into two subclasses, the classical PTPs and dual specific phosphatases (DUSPs). The classical PTPs contain both transmembrane receptor protein tyrosine phosphatases (PTPRs) and non-receptor protein tyrosine phosphatases (PTPNs). PTPRs are localized to plasma membrane and bind to ligand through their extra cellular domains and regulate cellular signaling. Many PTPRs are shown to function as cell adhesion molecules and also in processes such as cell–matrix and cell–cell contacts. Out of 21 PTPRs, 12 have a tandem arrangement of PTP domain in iwntracellular segment. The membrane-proximal (D1) domain is active whereas membrane-distal (D2) is inactive except for PTPalpha.[13] But D2 domain is required for the activity, stability, substrate specificity and also is important for PTPR dimerization.[14, 15] Non-receptor PTPs are cytosolic and contains extra regulatory sequences flanking the catalytic site, which control their activity through either masking the active site (in case of SHP2) or by defining substrate specificity (in case of PTP-PEST).[16, 17] These extra domains also regulate the sub cellular localization thus restricting the activity at specific locations.

Dual-specificity phosphatases (DUSPs) have short catalytic domains than classical PTPs but they have similar catalytic mechanism as the classical PTPs. The unique active site of DUSPs allows them to act on all three phosphorylated (Ser, Thr, and Tyr) residues. DUSPs are also known to recognize non-protein substrates such as mRNA and phosphoinositides.[18] One of the well-understood DUSP subfamily is MKP (MAP Kinase phosphatases). These MKPs inactivate the MAPKs by dephosphorylation of Thr and Tyr present in kinase activation site. MKPs localize to different subcellular compartments, differ in pattern of induction and specific for each MAPK, which are crucial in maintaining the MAPK signaling. MTMRs are inositol lipid phosphatases that constitute another subfamily of DUSPs. Out of 14 members known, only 8 are known to encode active enzymes and other 6 encode pseudo-phosphatases.[19, 20] All MTMRs contain an N-terminal PH-GRAM (Pleckstrin Homology-Glucosyltransferase, Rab-like GTPase Activators and Myotubularins) domain, a PTP catalytic domain (CSDGWDRT), and a C-terminal coiled-coil region. The PH-GRAM domain mediates the binding with phosphoinositides, mainly PtdIns5P and PtdIns(3,5)P2. Many MTMRs are known to be altered in human diseases, for example, MTM1 and MTMR2 are found to have mutated in X-linked myotubular myopathy and Charcot–Marie–Tooth disease type 4B (CMT4B), respectively; however physiological roles of MTMRs are still not well understood.[20]

Class II Cys-based PTPs contain a small group of CDC25 phosphatases, which are critical cell cycle regulators (described in other sections below). Although their catalytic activity is very similar to Class I PTPs, they are structurally unrelated. The class III Cys-based PTPs are widely present in all kingdoms of life and humans contain single gene for this class, a low-molecular-weight protein tyrosine phosphatase (LMPTP). Although genetic polymorphisms of this gene are known to be associated with several human diseases, including allergy, asthma, obesity, myocardial hypertrophy, and Alzheimer's disease, the functions of this phosphatase remain unclear.

Cell cycle: A sequence of events that occur in a cell with defined phases leading to its duplication of DNA and division into two daughter cells.

The fourth class of PTPs, the Asp-based phosphatases, includes HAD superfamily, exemplified by EYA (eyes absent) tyrosine phosphatases. This class of phosphatases mainly functions as transcription factors via binding to SIX proteins and are involved in development of kidney, muscle, eye, and ear.[21, 22] Eya family phosphatases are found to be mutated in many disorders, like, congenital cataracts, late-onset deafness, and the multi-organ disease bronchio-oto-renal syndrome.

Phosphatases play a critical role in cellular signaling by controlling both the temporal dynamics and spatial localization of phosphoproteins. They function as immediate as well as delayed regulators of protein phosphorylation, which often results in attenuation or propagation of signals. Immediate phosphatase activities

develop very rapidly on a scale of seconds to shape the initial phosphorylation profiles of receptors, ser/thr kinases, phosphorylated adaptors, and other signaling molecules activated by ligands such as growth factors. On the other hand, induced phosphatase activities occurring in the time scale of hours make cells adapt to permanent external cues by creating either negative or positive feedback loops. Here, in this review, we focus on some of the spatio-temporal control mechanisms exerted by phosphatases critical for the propagation of important cellular pathways.

3 Control of Insulin Signaling by Phosphatases

Insulin mediates its effects by activating the Insulin receptor (IR), a transmembrane protein tyrosine kinase that phosphorylates itself as well as target substrates such as IR substrate (IRS)-1 and -2.[23] Upon binding insulin, the IR undergoes transphosphorylation on several sites including the Y1162/Y1163 β-subunit PTK activation loop site. While Y1162/Y1163 phosphorylation is required for IR activation, the phosphorylation of other sites, including the juxta membrane Y972 site allows for the recruitment of IRS-1. IRS-1/2 tyrosine phosphorylation events, in turn, result in PI3K activation, which converts the lipid PIP2 (phosphatidylinositol (4, 5)-bisphosphate) to PIP3 (phosphatidylinositol 3, 4, 5-trisphosphate) followed by the activation of protein kinases including Akt that mediate the metabolic actions of insulin.[24] In particular, activated PI3K via Akt mediates the translocation of the GLUT4 transporter onto the plasma membrane for glucose uptake. Several phosphatases have been implicated in controlling the dynamics of insulin signaling (Fig. 2). Lipid and protein tyrosine phosphatase, phosphatase and tension homologue (PTEN), is one of the well-known negative regulators of insulin signaling. PTEN by dephosphorylating PIP3 to PIP2 controls the activation of insulin signaling. PTEN expression downregulates GLUT4 membrane levels and thus reduces insulin-induced glucose uptake. On the other hand, microinjection of an anti-PTEN antibody increased basal and insulin stimulated GLUT4 translocation, suggesting negative role of PTEN in controlling insulin pathway dynamics. In addition to lipid dephosphorylation, PTEN was also recently shown to control the insulin dynamics via acting as a tyrosine phosphatase for IRS-1.[25]

Among other phosphatases, Lar phosphatase (PTPRF) expression is found to be increased in fat tissues of obese patients and it also known to bind insulin receptor upon insulin treatment.[26, 27]

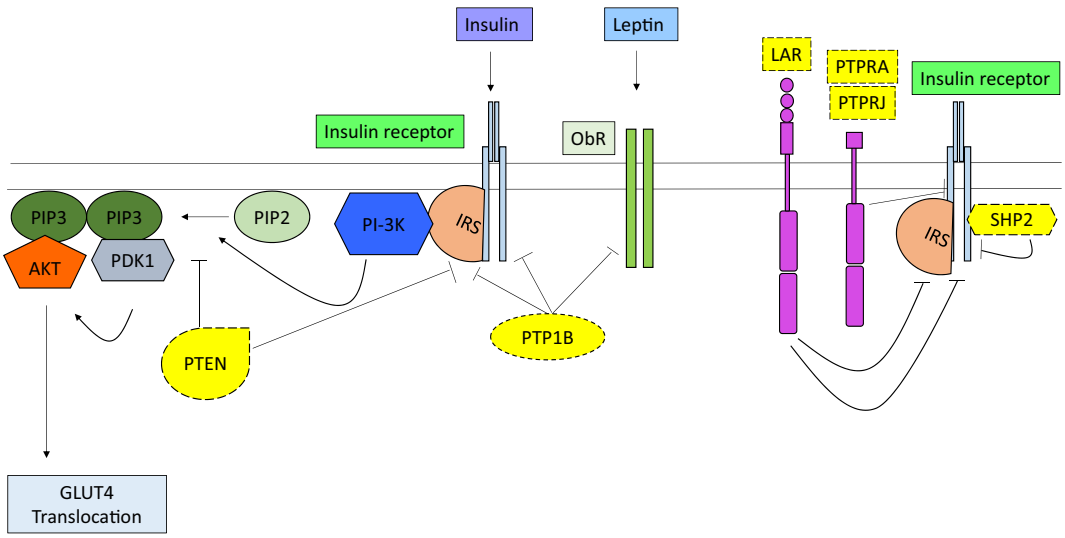

Figure 2: Protein phosphatases and insulin signaling. Upon binding to insulin receptor, insulin activates insulin-receptor kinase that leads to recruitment of IRS proteins to insulin receptor. IRS activates PI3K kinase to convert PIP2 to PIP3 which results in activation of PDK1 and AKT leading to GLUT4 translocation to plasma membrane and increased glucose uptake. Phosphatases PTEN, PTP1B, LAR, PTPRA, PTPRJ, and SHP2 known to regulate this pathway at different levels are shown by *dotted lines*. ***IRS*** insulin-receptor substrate, ***PI3K*** phosphatidylinositol 3-kinase, ***PIP2*** phosphatidylinositol 4,5-bisphosphate, ***PIP3*** phosphatidylinositol (3,4,5)-trisphosphate, ***PDK1*** phosphatidylinositol-dependent kinase 1, ***AKT***(***also known as PKB***) protein kinase B, ***SHP2*** SH2 domain containing protein tyrosine phosphatase-2.

GENE	Substrate specificity	localization
DUSP1	JNK >p38= ERK	Nucleus
DUSP2	ERK= p38>JNK	Nucleus
DUSP4	ERK =JNK> p38	Nucleus
DUSP5	ERK	Nucleus
DUSP6	ERK	Cytoplasm
DUSP7	ERK	Cytoplasm
DUSP9	ERK> p38	Cytoplasm
DUSP8	JNK= p38	Cytoplasm/Nucleus
DUSP10	JNK =p38	Cytoplasm/Nucleus
DUSP16	JNK =p38	Cytoplasm/Nucleus

Figure 3: Substrate specificity and differential localization of various MAPK phosphatases was shown.

Overexpression of Lar has been shown to suppress insulin signaling [28] and its depletion results in enhanced and prolonged insulin signaling.[29] Also, Lar overexpressed in mouse skeletal muscle shows insulin resistance through IRS-2.[30] A receptor tyrosine phosphatase PTPRA has also been shown to alter the dynamics of insulin signaling by dephosphorylation of insulin receptor.[31] Another related phosphatase DEP-1/PTPRJ also demonstrated to dephosphorylate insulin receptor[32] and the corresponding knockout mice showed enhanced insulin signaling.[33]

SHP-2/PTPN11 is an additional phosphatase demonstrated to modulate insulin signaling via associating with insulin receptor and IRS-1.[34, 35] The role of SHP-2 in insulin signaling has been controversial. While in vivo studies with SHP-2 dominant negative transgenic mice have shown insulin-resistant phenotype suggesting a positive role of phosphatase in insulin signaling,[36] other studies with SHP-2 knockout animal model could not support its role in insulin signaling where no defects were found in these mice model.[37] Function of SHP2 during insulin response might vary with different tissue types, and thus generation of tissue-specific knockout mice would allow to spatially and temporally define its role in insulin signaling.

Also, PTP1B/PTPN1 has a well-known role in insulin signaling, where it act as a negative regulator.[38, 39] PTP1B directly binds to IR and dephosphorylates both IR and IRS-1.[40–42] Homozygous PTP1B knockout mice had shown abnormal glucose and insulin tolerance, increased sensitivity to insulin, and increased phosphorylation of IR after insulin treatment.[43, 44] Together, involvement of multiple phosphatases highlights the importance of tight spatial and temporal control of insulin signaling.

4 Regulation of MAPK Signaling by MKP Phosphatases

MAPK signaling is a well-conserved pathway that is involved in various biological processes, such as metabolism, immunity, cell proliferation, and differentiation. Abnormalities in MAPK pathway is reported in many human diseases, such as diabetes, obesity, neurodegenerative disorders, cancer, and rheumatoid arthritis. MAPK pathway is comprised of three sequential protein kinases: MAPK kinase kinase (MAPKKK), MAPK kinase, and MAPK. MAPKKK is activated by extracellular signal through binding to a small GTPase or a kinase downstream of receptor. This activated MAPKKK directly phosphorylates MAPKK, which then activates MAPK by phosphorylating a T–X–Y motif in activation region.[45] MAPK can be divided into three groups, such as ERKs, JNKs, and p38/SAPKs. These groups of MAPK are activated by specific extracellular signal.[45]

The amplitude and duration of MAPK network is critical in determining the cellular responses. For example, a rapid and transient MAPK activation in rat hepatocytes promotes G1/S cell cycle progression, where as its constitutive activation results in inhibition of G1/S transition. Thus, by controlling MAPK dynamics via phosphorylation/dephosphorylation cycles, phosphatases ensure proper functioning of MAPK signaling cascades. MAPK phosphatases belong to a family of DUSPs that negatively regulate MAPK pathway by

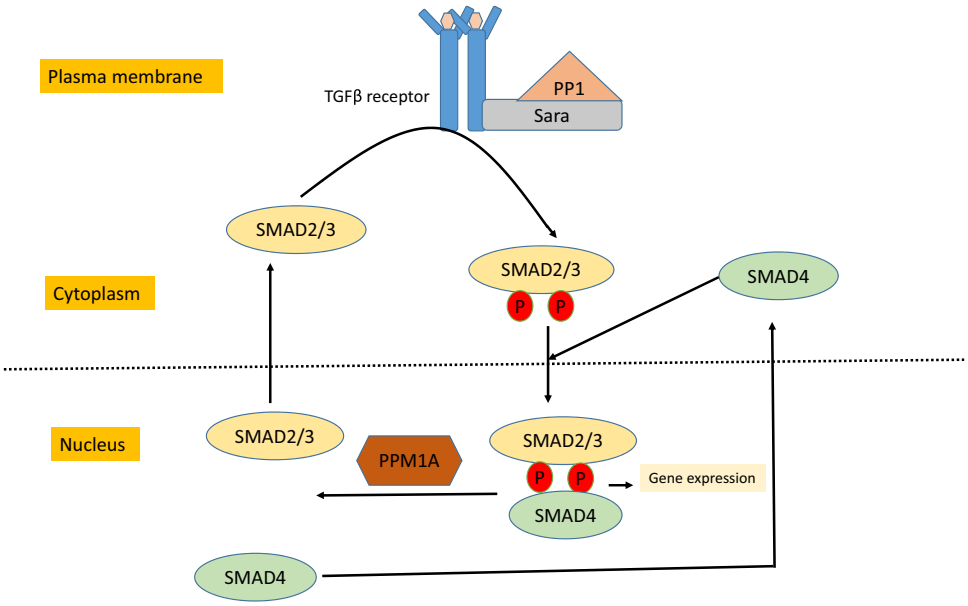

Figure 4: Phosphorylation dynamics in TGFβ signalling. TGFβ binding to its receptor kinases leads to phosphorylation of SMAD2/3. Phosphorylation of SMAD2/3 allows binding with SMAD4 and nuclear translocation where this complex binds to various transcription cofactors that regulate gene expression of many genes. This signalling in nucleus is inhibited by PPM1A phosphatase that dephosphorylates SMAD2/3 that results in dissociation of SMAD2/3–SMAD4 complex and both proteins are exported out of nucleus. At plasma membrane, sara recruits PP1 phosphatase which dephosphorylates TGFβ receptor and signal inhibition.

directly dephosphorylating T–X–Y motif in activation loop of kinase.[46] The MAPK phosphatases (MKP) are classified into three subgroups based on sequence homology, subcellular localisation, and substrate specificity. While there are four inducible nuclear MKPs (*DUSP1*, *DUSP2*, *DUSP4,* and *DUSP5)*, three are cytoplasmic MKPs (*DUSP6*, *DUSP7,* and *DUSP9*) and further three MKPs (*DUSP8*, *DUSP10*, and *DUSP16*) are distributed in cytoplasm as well as nucleus. These MAPK phosphatases localize to different cell compartments and show distinct substrate preference (Fig. 3). In addition to their dephosphorylating function, DUSPs also regulate subcellular localization of MAPKs. For instance, DUSP16 by virtue of its NLS and NES transports p38 and JNK from nucleus to cytoplasm. Similarly, DUSP6 helps in the retention of ERK2 in the cytoplasm. Interestingly, DUSP activity is regulated by substrates where binding of MAPK enhances DUSP activity. In addition, many DUSPs themselves are found to be substrates of MAPK and therefore contribute to the cellular feedback mechanism. These feedback regulators are required to respond rapidly and with high precision to changes in MAPK activity. The feedback control by DUSPs shapes the dynamics of mitogenic responses. Several studies demonstrated that negative feedback control of Ras/ERK signaling by MKPs may play an important role in determining the biological outcome of signaling when upstream components of this pathway, such as receptor tyrosine kinases (RTKs), Ras isoforms or Braf, are mutated and activated. The fact that MAPK signaling is often abnormally activated in human cancers suggests that DUSPs may also be regulated as a result of the oncogenic activation of MAPK signaling. This is supported by numerous observations of either increased or decreased MKP expression in malignant disease, suggesting that these enzymes might play important role in cancer initiation and/or progression.

5 Phosphatases in TGFbeta and BMP Signaling

TGFbeta signaling has well-known role in cell differentiation, proliferation, and development (Fig. 4). TGF ligands bind to its receptor kinase at plasma membrane that results in oligomerization of receptors and phosphorylation of cytoplasmic proteins SMAD2/3 for TGF and SMAD1/5/8 for BMP signaling. This phosphorylation of C-terminal SXS motif allows binding with MH2 domain of SMAD4 and leads to nuclear translocation of

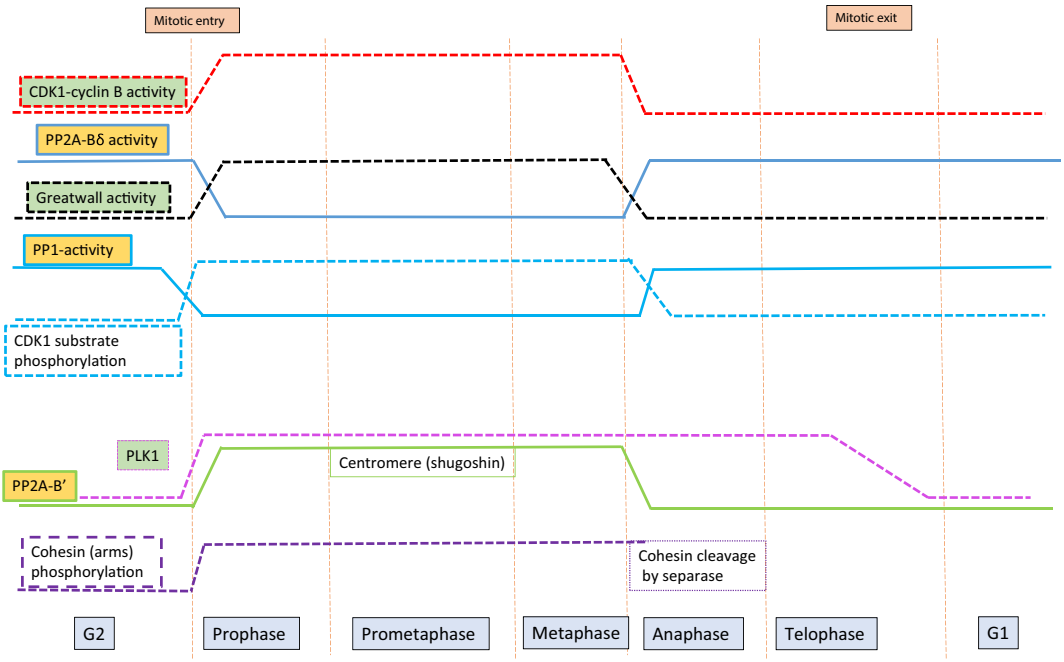

Figure 5: Dynamic activity changes of selected kinases and phosphatases during mitosis. Mitotic entry is characterized by heavy phosphorylation due to high kinase/phosphatase activity ratio but at the exit the activities are reversed and thus resulting in ordered dephosphorylation of proteins.

this complex where it regulates gene expression. Thus, dephosphorylation of SMAD proteins may be necessary to control TGF signaling.[47] To identify SMAD2/3-specific phosphatases, Lin et al.[48] screened 39 phosphatases and demonstrated only PPM1A was able to dephosphorylate C-terminal site of SMAD2/3. They also showed PPM1A binds directly to SMAD2/3 and has more affinity for phosphorylated SMAD2/3 compared to non-phosphorylated SMAD2/3. PPM1A is localized to nucleus and dephosphorylates the SMAD2/3 upon TGF stimulation that results in nuclear

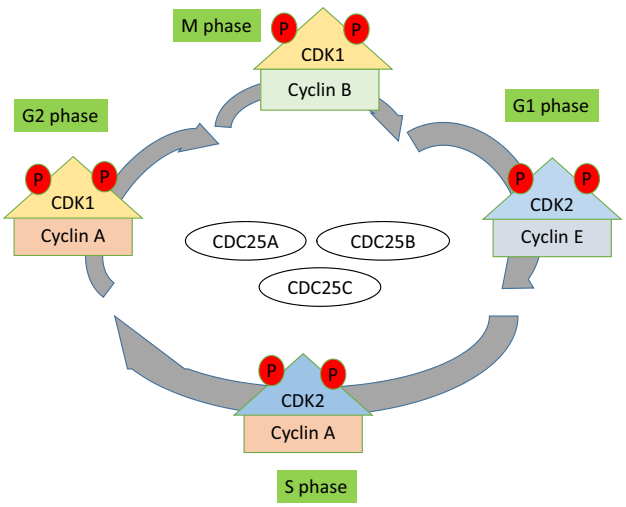

Figure 6: CDC25 phosphatases regulate cell cycle transitions. All three isoforms of CDC25 phosphatase, CDC25A, CDC25B, CDC25C, are involved in dephosphorylation of CDK–cyclin complexes, such as CDK1–cyclin B dephosphorylation during mitosis entry and CDK2–cyclin E dephosphorylation at G1–S transition.

export of SMAD2/3. Furthermore, another study has also found PP1-binding protein Sara to target PP1 to TGF receptors to dephosphorylate it and terminate TGF signaling.[49] On the other hand, two independent studies reported that SCP1-3 phosphatases (not FCP1) enhance TGF signaling by dephosphorylating the linker regions in SMAD2/3.[50, 51]

BMP signaling functions similarly to TGF signaling but instead of SMAD2/3, BMP ligands induce phosphorylation of SMAD1/5/8 which in turn leads to binding to SMAD4 and entry into nucleus. An siRNA-based screening using drosophila S2 cells identified PDP phosphatase that dephosphorylates SMAD1, which was later confirmed in human cells as well.[52] Also, PDP phosphatase was found to have no effect on SMAD2/3 phosphorylation. Interestingly, SCP1-3 phosphatase-mediated dephosphorylation of SMAD1 was shown to attenuate BMP signaling.[53] Consequently, while SCP1/2 knockdown inhibited TGFbeta transcriptional responses, but it enhanced BMP transcriptional responses. Thus, context-based activation of phosphatases

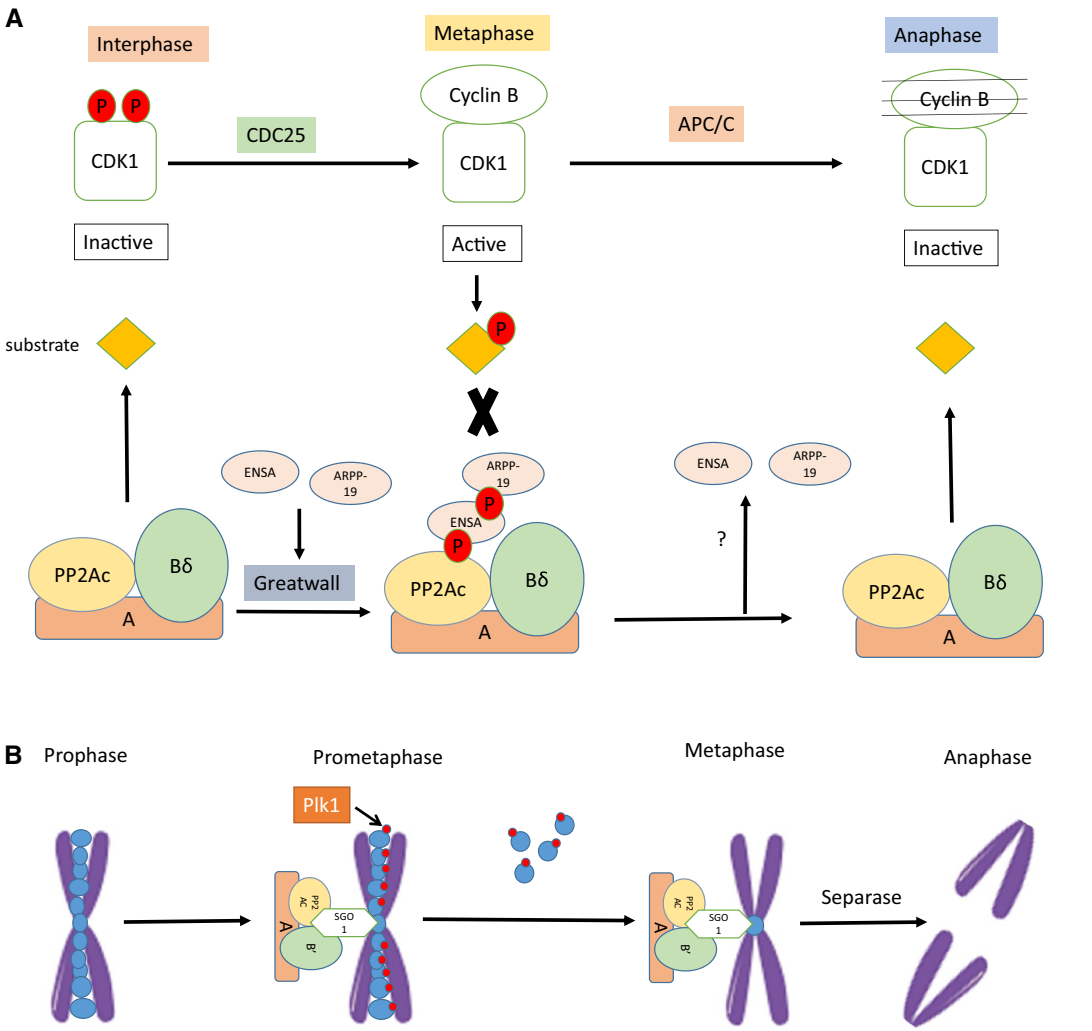

Figure 7: PP2A dynamics in mitosis: **a** during interphase CDK1 is kept inactive by inhibitory phosphorylation and PP2A-Bδ is active. During G2–M transition, CDC25 dephosphorylate CDK1 that leads to association with cyclin B. activated CDK1–cyclin B activates Greatwall kinase which phosphorylates PP2A-Bδ inhibitory proteins ENSA and ARPP-19 and inactivate PP2A-Bδ. Phosphorylated forms of CDK1 substrates accumulate in metaphase. During transition from metaphase to anaphase, APC degrades CDK1 that leads to dissociation of ENSA and ARPP-19 and CDK1 substrate are dephosphorylated by PP2A-Bδ. **b** During prophase, Plk1 phosphorylate cohesin complex that dissociates cohesin from chromosomes except centromeric region. Sgo1 recruits a local pool of PP2A-B′ on centromeres that keep cohesin in dephosphorylation forms and counteracts the Plk1 function.

followed by dephosphorylation of distinct substrates is proven to be critical for appropriate TGF beta/BMP responses.

Together, it is becoming clear that phosphatase-mediated deactivation of cellular pathways is used by cells to control different aspects of signaling, be it the duration, amplitude or localization of the signal.

6 Protein Phosphatases and Cell Cycle

In addition to cellular signaling pathways, phosphatases actively participate in the spatio-temporal control of cell cycle, in particular mitosis. The timely (de)phosphorylation of hundreds of proteins contributes to the intricate control of mitotic transition through different phases (Fig. 5). For instance, at the beginning of mitosis, high magnitude of phosphorylation generally mediated by kinases, such as CDK1, Plk1, Greatwall kinase, and Aurora A, is observed. During mitotic entry, as counteracting phosphatases largely remain inactive, higher phosphorylation stoichiometry of proteins was achieved at this stage. In contrast, the mitotic exit is characterized by an ordered bulk dephosphorylation of phosphoproteins, due to drastically increased phosphatase/kinase activity ratios. On the other hand, the dynamic phosphorylation during intra mitotic phases is associated with low phosphorylation stoichiometries since some of the kinases and phosphatases (such as Plk1 and PP2A-B′) are simultaneously active, although not necessarily at the same location. The details of some of the time and location-specific dynamic (de)phosphorylation events that occur during mitosis are discussed in the sections below. CDK protein complexes are important kinase regulators of cell cycle and these CDK complexes are kept in inactive state by phosphorylation of their ATP loop by MYT1 and WEE1 kinases.[54] CDC phosphatases activate CDK–cyclin complexes by dephosphorylating these inhibitory phosphorylation sites and thus facilitate entry into next phase of cell cycle.[55, 56] CDC phosphatase family contains three isoforms and regulates the G1–S and G2–M transition by controlling the activities of CDK2 and CDK1, respectively (Fig. 6). CDC25A regulates the G1-S transition by activating CDK2–cyclin E and CDK2–cyclin A.[57–59] CDC25A is also involved in G2–M transition through activation of CDK1–cyclin B complex which initiates chromosome condensation.[60–62] CDC25B is responsible for initial activation of CDK1–cyclin B on centrosomes during G2–M transition[63, 64] and CDC25C fully activates it in nucleus during start of mitosis.[65] Studies using RNAi knockdown also demonstrated the role of CDC25B/CDC25C in S-phase entry.[66, 67] All three CDC phosphatases spatially and temporally activate their substrates and function as crucial regulators of G1–S and G2–M transition.

Among different phases of cell cycle, mitosis is highly subjected to control via phospho-dephosphorylation cycles, which is discussed in detail below. Many reports have suggested that activation of CDK by dephosphorylation is not sufficient for normal mitosis and there exists an okadaic acid-sensitive phosphatase that acting on CDK substrates needs to be inhibited for entry into mitosis.[68] Furthermore, this phosphatase activity is also required for mitotic exit.[69] PP2A was identified to be the phosphatase which makes cells more sensitive to CDK1 activation and promote the mitotic entry.[70–74] Greatwall kinase phosphorylates two small heat stable proteins endosulfine (ENSA) and c-AMP-regulated phosphoprotein-19 (ARPP-19), and these phosphorylated protein binds to PP2A holoenzyme containing PPP2R2D (B delta) regulatory subunit and leads to inhibition of PP2A-b delta phosphatase complex.[75, 76] PP2A-B delta phosphatase is highly active in interphase and less active in mitosis, so its activity is the inverse of CDK1–cyclin B activity (Fig. 7a).

6.1 Phosphatases During Cohesion Control in Mitosis

During mitosis, chromosomes are formed by DNA condensation and it results in formation of sister chromatids. These sister chromatids are joined to each other through a multisubunit protein complex known as cohesin that forms a ring-like structure around the sister chromatids.[77] During prophase, most of cohesin is removed from the chromosomes by Plk1 and aurora B kinase-mediated phosphorylation of cohesin complex.[78] However, cohesin complex at centromere regions stays protected against Plk1 and Aurora B and provides a unique X-shaped morphology to chromosomes. This X-shape is crucial for attachment of spindle to chromosomes to ensure proper sister chromatid separation. Cohesin at centromere region is protected by Shugoshin 1 (sgo1). Shugoshin 1 was initially identified in yeast that is required to protect cohesin Rec8 during meiosis.[79] Shugoshin 2 (sgo2), a Sgo1 paralog, has been shown to have similar role in mitosis. In humans, two shugoshins are known—sgo1 and sgo2. Sgo1 localizes to centromere and its depletion results in loss

Mitosis: A particular phase of the cell cycle where duplicated DNA is separated into two nuclei followed by separation of a mother cell into two daughter cells by division.

Spindle: A cytoskeletal structure formed by group of spindle fibres containing tubulin and associated proteins that pull the chromosomes towards opposite poles during cell division.

Centromere: A part of the chromosome that attaches to spindle fibres during cell division through assembly of multiprotein structure called kinetochores.

Centrosome: A cellular organelle from which spindle fibres develop during cell division.

of sister chromatid cohesion in early mitosis whereas Sgo2 was suggested to have role in meiosis not for mitosis.[80–82] In sgo1 knockdown cells, a non-phosphorylated form of cohesin subunit (SA2) was able to rescue the loss of sister chromatid phenotype.[81] Later, it was shown that sgo1 recruits PP2A holoenzymes containing B′ subunits to centromere and keeps the Plk1 and aurora substrate (SA2) in dephosphorylated state.[83–85] Therefore, non-phosphorylated forms of cohesin at centromere are not removed during prophase whereas phosphorylated cohesin is removed from chromosomes arms (Fig. 7b).

6.2 Phosphatase Action During Spindle Formation

A stable bipolar spindle formation is crucial for proper segregation of chromosomes during mitosis.[86] First step in this process is centrosome duplication and separation that yields two microtubule-organizing centers. Nek2 protein kinase controls this centrosome-splitting process and a PP1 family phosphatase plays an opposite role.[87–89] PP1gamma isoforms interact with Nek2 by binding to its RVxF like motif, and regulate its kinase activity and also dephosphorylates Nek2 substrate such as centriolar linker protein C-nap1.[87] Other reports have also showed both PP1alpha and PP1gamma interact with Nek2, but PP1alpha was found to be more crucial for Nek2 kinase activity regulation in vivo.[89] After centrosome separation, centrosomes move away to form microtubule-organizing centers that further leads to formation of bipolar spindle. This event is regulated by two protein kinases—Aurora A and Plk1. Disruption of either of these kinases leads to formation of monopolar spindles and failure of chromosome segregation.[90, 91] Activity of both Aurora A and Plk1 is regulated by phosphorylation of T-loop or activation loop at Thr residue. Aurora A and related Aurora B kinase are targeted by binding to TPX2 and INCEP protein, respectively, that keeps T-loop phosphorylation protected from phosphatases and stabilizes the active form.[92–94] It is believed that at start of mitosis these kinases are activated by T-loop phosphorylation and are degraded at end of mitosis. But activity of these kinases is tightly regulated both spatially and temporally at various sites and at different times during mitosis. So, a dynamic equilibrium exists between the phosphorylated and dephosphorylated form of T-loop. PP1 and PP2A are shown to dephosphorylate Aurora T-loop in vitro and implicated to regulate it. Recently Zeng et al. demonstrated that PP6 phosphatase is a major T-loop phosphatase in mitotic cells.[95] Furthermore, only depletion of PP6 catalytic or regulatory subunits, but not PP1 or PP2A, results in increase in the T-loop phosphorylation. In absence of PP6, Aurora A kinase becomes hyperactive that yield to impaired bipolar spindle assembly and chromosome segregation.

Aurora B kinase is also a key player in mitosis that is involved in bipolar spindle attachment to chromosomes. Like Aurora A, Aurora B is regulated by its T-loop phosphorylation and interacts with INCENP. INCENP binding is necessary for Aurora B activation and chromosome localization.[96] PP1-PPP1R7 holoenzyme complex has been demonstrated as T-loop phosphatase and cells depleted of this complex showed defects in chromosome attachment to spindles.[97, 98] But complete mechanism of PP1-PPP1R7-meditated dephosphorylation is still unclear. Also, how both PP6 and PP1-PPP1R7 dephosphorylate a site in T-loop that buried inside the kinase active site is still not known. To address this problem, structure of phosphatases bound to its substrate may need to be resolved. Another PP1 holoenzyme, PP1gamma, is shown to dephosphorylate the substrate of Aurora B kinase and stabilizes the kinetochore–microtubule attachment under tension.[99] PP1gamma binds directly to RVXF and SILK docking motif in the KNL1 subunit of KMN complex.[99–101] Under no tension state, Aurora B is in close proximity to KNL1, phosphorylate it near to PP1 docking site that inhibits the binding of PP1gamma. This results in phosphorylation of Aurora B substrate and destabilization of microtubule attachments. Under tension, kinetochore is stretched away from centromere, so KNL1 does not get phosphorylated by Aurora B. PP1 can interact with KNL1 and dephosphorylate kinetochore substrate of Aurora B that results in stabilization of microtubule attachment to kinetochore.

6.3 Phosphatase Dynamics During Mitotic Exit

Once all chromosomes are properly aligned at metaphasic plate and spindle checkpoint is cleared CDK1 phosphorylates APC/C ubiquitin ligase. This leads to binding of APC to its coactivator cdc20 that targets many mitotic substrates and initiates mitotic exit. Spindle assembly checkpoint keeps APC in inactive state till all chromosomes are properly attached to spindles. Mitotic exit includes all events that occur after the spindle assembly checkpoint including chromosome

segregation, cytokinesis, and assembly of interphase cell structures. APC ubiquitinate two important proteins—cyclin B and the separase inhibitor securin. Cyclin B degradation results in inactivation of CDK1 thus promoting transition from metaphase to anaphase. Securin degradation activates separase that leads to cohesin removal from centromere and results in sister chromatid separation.[102] During late stages of anaphase due to low activity of CDK1, APC binds to its second coactivator cdc20 homologue 1 (CDH1) which enables APC to target CDC20, Aurora kinases, and PLK1. But phosphorylation by CDK1 and other kinases still need to be removed for exit from mitosis. Cdc14 acts as main mitotic exit phosphatase in budding yeast.[103] But role of Cdc14 phosphatase in mammalian cells is still elusive although Cdc14 has been shown to be involved in anaphase and cytokinesis regulation.[104] Thus, it may not be the major phosphatase to dephosphorylate the CDK1 substrates. Moreover, Cdc14 has been demonstrated to have more important role in DNA damage repair than mitotic exit regulation.[105] In mammalian cells, the phosphatases required for mitotic exit are not fully understood but some recent studies have provide few clues on role of PP2A family and PP1 phosphatases during this process. PP2A-B55 targets CDK1 consensus site on CDK1 substrates (Ser-Pro or Thr-Pro) in vitro whereas regulatory subunits of other phosphatase do not confer specificity to CDK1 substrate.[106, 107] In drosophila, mutation in PP2A-B55 subunit results in abnormal chromosome segregation in anaphase.[108] This suggests a crucial role of PP2A-B55 in regulating animal cell mitotic exit. Further, biochemical studies in *Xenopus* embryonic extracts and RNAi screening in human cell lines have confirmed the role of PP2A-B55 as regulator of mitotic exit.[109, 110] In human cells, depletion of B55alpha results in delayed formation of nuclear membrane and Golgi apparatus along with delayed disassembly of spindles and chromosome decondensation. Using CDK1 inhibitor to induce mitotic exit also demonstrated that PP2A-b55alpha act on CDK1 substrates but not on CDK1 itself.[110]

PP1 is another proposed Ser/Thr phosphatase that dephosphorylates CDK1 substrates and helps in mitotic exit in animal cells. Studies in drosophila have suggested that depletion of PP1 induces chromosome missegregation and abnormal spindle in anaphase.[111, 112] Furthermore, immunodepletion of PP1 or addition of PP1 Inhibitor 1 results in delayed CDK1 substrate dephosphorylation in *Xenopus* egg extracts.[113] Also, its depletion in mouse fibroblasts shows premature CDK1 substrate dephosphorylation. PP1-repo-men (CDCA2) is involved in chromatin architecture maintenance during anaphase and negatively regulated by CDK1–cyclin B by direct phosphorylation till anaphase onset.[114, 115] Then, repo-men become dephosphorylated and recruits PP1gamma to chromatin. A recent study has demonstrated histone H3 as substrate of PP1-repo-men.[116] H3 is phosphorylated by kinase haspin at Thr 3 position and mostly found at centromeres, where it helps in binding to Aurora B chromosome passenger complex.[117, 118] Thus, a locally repo-men-bound pool of PP1gamma dephosphorylates H3 during metaphase and during mitotic exit and directs Aurora B away from centromere toward the chromosome arms.[116]

Cytokinesis: A process of cytoplasmic division of a cell at the end of mitosis or meiosis that leads to division into two daughter cells.

6.4 Phosphatase Requirement During Rebuilding of Interphase Cell

During mitosis entry, nuclear envelope breakdown is initiated by CDK1-mediated phosphorylation of lamin proteins and nucleoporins.[119] PP1 and PP2A phosphatases are necessary for reassembly of nuclear envelope but it is not clear whether these phosphatases act directly on lamin or nucleoporin proteins.[120, 121] PNUTS, a PP1 regulatory subunit, localizes to chromatin after nuclear envelope formation and helps in chromosome decondensation.[122] PP2A-Balpha (PPP2R2A) is involved in assembly of Golgi apparatus during mitotic exit. Golgi apparatus is disassembled by phosphorylation of Golgi matrix protein GM130 and Golgi stacking proteins by CDK1.[123] During anaphase, PP2A-B alpha dephosphorylates GM130 at CDK1 phosphorylation site that leads to tethering of Golgi vesicles and self-organization to form stacked Golgi apparatus in interphase.[124] Thus, taken together, a tight spatial and temporal control exerted by phosphatases at different phases of cell cycle, in particular mitosis is critical for maintenance of genomic integrity in the cell.

7 Concluding Remarks

The field of phosphatases has come a long way since their discovery. Earlier phosphatases were considered as housekeeping enzymes which were unresponsive to hormone signaling and therefore considered as physiologically uninteresting. Now role of phosphatases is well appreciated in many biological signaling pathways and human diseases. For many years phosphatases were considered as non-specific promiscuous enzymes with no or little substrate specificity

especially Ser/Thr phosphatases. But, now, it is clear that Ser/Thr phosphatases form complexes with distinct regulatory subunits and target-specific proteins.[125] Although many studies have reported a catalog of regulatory subunits for PPP phosphatases but function of only a handful of these regulatory subunits are known and majority of regulatory subunits and their substrate are still unknown. Next, challenge in the field is to find the substrates of every phosphatase and their phosphorylation site on each of these targets. Mass spectrometry-based proteomics can be used to identify the components of phosphatase complexes and to determine potential substrates of phosphatase. Recently, some studies have used affinity purification coupled with mass spectrometry to identify proteins associated with phosphatases.[126, 127] But, role of many of these novel protein interaction identified through AP-MS are still poorly understood. Substrate trapping mutants of phosphatases can be a feasible way to identify the substrates, which are normally very sensitive for identification owing to their transient association with active phosphatases. In one such example, recently, we utilized PTEN trap mutant to identify novel substrates which were not found earlier using active enzymes. We identified Rab7 as a novel substrate of PTEN and functionally demonstrated that PTEN inhibits EGFR signaling by promoting early to late endosome transition.[128] On the other hand, recent advances such as genome-wide high-throughput RNAi and knockouts using CRISPR method can be utilized to understand the function of phosphatases in a particular biological process. Many phosphatases have been linked to human diseases including cancer, thus targeting these phosphatases to disrupt their binding to substrate or with specific regulatory subunits in case of PPP phosphatases could be of potential therapeutic value. However, roles of majority of phosphatases in development and diseases in vivo are not fully understood. This will need development of conditional or tissue-specific knockouts of different phosphatases to explore their function. Further, phosphatases so far have been studied as individual enzymes. However, given the complex combinatorial nature of cellular signaling controlled by phosphatases and possibly multiple-associated proteins highlights the necessity of systems biology-based approaches in immediate future to understand the role of phosphatases in signaling dynamics and further targeting in drug development.

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References

1. Manning G, Whyte DB, Martinez R, Hunter T, Sudarsanam S (2002) The protein kinase complement of the human genome. Science 298:1912–1934
2. Manning G, Plowman GD, Hunter T, Sudarsanam S (2002) Evolution of protein kinase signaling from yeast to man. Trends Biochem Sci 27:514–520
3. Cohen P (2002) The origins of protein phosphorylation. Nat Cell Biol 4:E127–E130
4. Cohen P (1994) The discovery of protein phosphatases: from chaos and confusion to an understanding of their role in cell regulation and human disease. BioEssays 16:583–588
5. Heinrich R, Neel BG, Rapoport TA (2002) Mathematical models of protein kinase signal transduction. Mol Cell 9:957–970
6. Hornberg JJ, Bruggeman FJ, Binder B, Geest CR, de Vaate AJ, Lankelma J et al (2005) Principles behind the multifarious control of signal transduction. ERK phosphorylation and kinase/phosphatase control. FEBS J 272:244–258
7. Das AK, Helps NR, Cohen PT, Barford D (1996) Crystal structure of the protein serine/threonine phosphatase 2C at 2.0 A resolution. Embo J 15:6798–6809
8. Schweighofer A, Hirt H, Meskiene I (2004) Plant PP2C phosphatases: emerging functions in stress signaling. Trends Plant Sci 9:236–243
9. Cho US, Xu W (2007) Crystal structure of a protein phosphatase 2A heterotrimeric holoenzyme. Nature 445:53–57
10. Xu Y, Xing Y, Chen Y, Chao Y, Lin Z, Fan E et al (2006) Structure of the protein phosphatase 2A holoenzyme. Cell 127:1239–1251
11. Archambault J, Pan G, Dahmus GK, Cartier M, Marshall N, Zhang S et al (1998) FCP1, the RAP74-interacting subunit of a human protein phosphatase that dephosphorylates the carboxyl-terminal domain of RNA polymerase IIO. J Biol Chem 273:27593–27601
12. Meinhart A, Kamenski T, Hoeppner S, Baumli S, Cramer P (2005) A structural perspective of CTD function. Genes Dev 19:1401–1415
13. Buist A, Zhang YL, Keng YF, Wu L, Zhang ZY, den Hertog J (1999) Restoration of potent protein-tyrosine

phosphatase activity into the membrane-distal domain of receptor protein-tyrosine phosphatase alpha. Biochemistry 38:914–922
14. Blanchetot C, Tertoolen LG, Overvoorde J, den Hertog J (2002) Intra- and intermolecular interactions between intracellular domains of receptor protein-tyrosine phosphatases. J Biol Chem 277:47263–47269
15. Jiang G, den Hertog J, Hunter T (2000) Receptor-like protein tyrosine phosphatase alpha homodimerizes on the cell surface. Mol Cell Biol 20:5917–5929
16. Garton AJ, Burnham MR, Bouton AH, Tonks NK (1997) Association of PTP-PEST with the SH3 domain of p130cas; a novel mechanism of protein tyrosine phosphatase substrate recognition. Oncogene 15:877–885
17. Pulido R, Zuniga A, Ullrich A (1998) PTP-SL and STEP protein tyrosine phosphatases regulate the activation of the extracellular signal-regulated kinases ERK1 and ERK2 by association through a kinase interaction motif. EMBO J 17:7337–7350
18. Mustelin T (2007) A brief introduction to the protein phosphatase families. Methods Mol Biol 365:9–22
19. Begley MJ, Dixon JE (2005) The structure and regulation of myotubularin phosphatases. Curr Opin Struct Biol 15:614–620
20. Laporte J, Bedez F, Bolino A, Mandel JL (2003) Myotubularins, a large disease-associated family of cooperating catalytically active and inactive phosphoinositides phosphatases. Hum Mol Genet 12(2):R285–R292
21. Li X, Oghi KA, Zhang J, Krones A, Bush KT, Glass CK et al (2003) Eya protein phosphatase activity regulates Six1-Dach-Eya transcriptional effects in mammalian organogenesis. Nature 426:247–254
22. Tootle TL, Silver SJ, Davies EL, Newman V, Latek RR, Mills IA et al (2003) The transcription factor Eyes absent is a protein tyrosine phosphatase. Nature 426:299–302
23. Sun XJ, Rothenberg P, Kahn CR, Backer JM, Araki E, Wilden PA et al (1991) Structure of the insulin receptor substrate IRS-1 defines a unique signal transduction protein. Nature 352:73–77
24. Saltiel AR, Pessin JE (2002) Insulin signaling pathways in time and space. Trends Cell Biol 12:65–71
25. Shi Y, Wang J, Chandarlapaty S, Cross J, Thompson C, Rosen N et al (2014) PTEN is a protein tyrosine phosphatase for IRS1. Nat Struct Mol Biol 21:522–527
26. Ahmad F, Considine RV, Goldstein BJ (1995) Increased abundance of the receptor-type protein-tyrosine phosphatase LAR accounts for the elevated insulin receptor dephosphorylating activity in adipose tissue of obese human subjects. J Clin Invest 95:2806–2812
27. Ahmad F, Goldstein BJ (1997) Functional association between the insulin receptor and the transmembrane protein-tyrosine phosphatase LAR in intact cells. J Biol Chem 272:448–457
28. Zhang WR, Li PM, Oswald MA, Goldstein BJ (1996) Modulation of insulin signal transduction by eutopic overexpression of the receptor-type protein-tyrosine phosphatase LAR. Mol Endocrinol 10:575–584
29. Kulas DT, Zhang WR, Goldstein BJ, Furlanetto RW, Mooney RA (1995) Insulin receptor signaling is augmented by antisense inhibition of the protein tyrosine phosphatase LAR. J Biol Chem 270:2435–2438
30. Zabolotny JM, Kim YB, Peroni OD, Kim JK, Pani MA, Boss O et al (2001) Overexpression of the LAR (leukocyte antigen-related) protein-tyrosine phosphatase in muscle causes insulin resistance. Proc Natl Acad Sci USA 98:5187–5192
31. Lammers R, Moller NP, Ullrich A (1997) The transmembrane protein tyrosine phosphatase alpha dephosphorylates the insulin receptor in intact cells. FEBS Lett 404:37–40
32. Andersen JN, Elson A, Lammers R, Romer J, Clausen JT, Moller KB et al (2001) Comparative study of protein tyrosine phosphatase-epsilon isoforms: membrane localization confers specificity in cellular signalling. Biochem J 354:581–590
33. Kruger J, Brachs S, Trappiel M, Kintscher U, Meyborg H, Wellnhofer E et al (2015) Enhanced insulin signaling in density-enhanced phosphatase-1 (DEP-1) knockout mice. Mol Metab 4:325–336
34. Rocchi S, Tartare-Deckert S, Sawka-Verhelle D, Gamha A, van Obberghen E (1996) Interaction of SH2-containing protein tyrosine phosphatase 2 with the insulin receptor and the insulin-like growth factor-I receptor: studies of the domains involved using the yeast two-hybrid system. Endocrinology 137:4944–4952
35. Kharitonenkov A, Schnekenburger J, Chen Z, Knyazev P, Ali S, Zwick E et al (1995) Adapter function of protein-tyrosine phosphatase 1D in insulin receptor/insulin receptor substrate-1 interaction. J Biol Chem 270:29189–29193
36. Maegawa H, Hasegawa M, Sugai S, Obata T, Ugi S, Morino K et al (1999) Expression of a dominant negative SHP-2 in transgenic mice induces insulin resistance. J Biol Chem 274:30236–30243
37. Arrandale JM, Gore-Willse A, Rocks S, Ren JM, Zhu J, Davis A et al (1996) Insulin signaling in mice expressing reduced levels of Syp. J Biol Chem 271:21353–21358
38. Cheng A, Uetani N, Simoncic PD, Chaubey VP, Lee-Loy A, McGlade CJ et al (2002) Attenuation of leptin action and regulation of obesity by protein tyrosine phosphatase 1B. Dev Cell 2:497–503
39. Asante-Appiah E, Kennedy BP (2003) Protein tyrosine phosphatases: the quest for negative regulators of insulin action. Am J Physiol Endocrinol Metab 284:E663–E670
40. Bandyopadhyay D, Kusari A, Kenner KA, Liu F, Chernoff J, Gustafson TA et al (1997) Protein-tyrosine phosphatase 1B complexes with the insulin receptor in vivo

and is tyrosine-phosphorylated in the presence of insulin. J Biol Chem 272:1639–1645
41. Dadke S, Kusari J, Chernoff J (2000) Down-regulation of insulin signaling by protein-tyrosine phosphatase 1B is mediated by an N-terminal binding region. J Biol Chem 275:23642–23647
42. Salmeen A, Andersen JN, Myers MP, Tonks NK, Barford D (2000) Molecular basis for the dephosphorylation of the activation segment of the insulin receptor by protein tyrosine phosphatase 1B. Mol Cell 6:1401–1412
43. Klaman LD, Boss O, Peroni OD, Kim JK, Martino JL, Zabolotny JM et al (2000) Increased energy expenditure, decreased adiposity, and tissue-specific insulin sensitivity in protein-tyrosine phosphatase 1B-deficient mice. Mol Cell Biol 20:5479–5489
44. Elchebly M, Payette P, Michaliszyn E, Cromlish W, Collins S, Loy AL et al (1999) Increased insulin sensitivity and obesity resistance in mice lacking the protein tyrosine phosphatase-1B gene. Science 283:1544–1548
45. Morrison DK (2012) MAP kinase pathways. Cold Spring Harb Perspect Biol 4(11):a011254. doi:10.1101/cshperspect.a011254
46. Caunt CJ, Keyse SM (2013) Dual-specificity MAP kinase phosphatases (MKPs): shaping the outcome of MAP kinase signalling. FEBS J 280:489–504
47. Massague J, Seoane J, Wotton D (2005) Smad transcription factors. Genes Dev 19:2783–2810
48. Lin X, Duan X, Liang YY, Su Y, Wrighton KH, Long J et al (2006) PPM1A functions as a Smad phosphatase to terminate TGFbeta signaling. Cell 125:915–928
49. Bennett D, Alphey L (2002) PP1 binds Sara and negatively regulates Dpp signaling in *Drosophila melanogaster*. Nat Genet 31:419–423
50. Sapkota G, Knockaert M, Alarcon C, Montalvo E, Brivanlou AH, Massague J (2006) Dephosphorylation of the linker regions of Smad1 and Smad2/3 by small C-terminal domain phosphatases has distinct outcomes for bone morphogenetic protein and transforming growth factor-beta pathways. J Biol Chem 281:40412–40419
51. Wrighton KH, Willis D, Long J, Liu F, Lin X, Feng XH (2006) Small C-terminal domain phosphatases dephosphorylate the regulatory linker regions of Smad2 and Smad3 to enhance transforming growth factor-beta signaling. J Biol Chem 281:38365–38375
52. Chen HB, Shen J, Ip YT, Xu L (2006) Identification of phosphatases for Smad in the BMP/DPP pathway. Genes Dev 20:648–653
53. Knockaert M, Sapkota G, Alarcon C, Massague J, Brivanlou AH (2006) Unique players in the BMP pathway: small C-terminal domain phosphatases dephosphorylate Smad1 to attenuate BMP signaling. Proc Natl Acad Sci USA 103:11940–11945
54. Mueller PR, Coleman TR, Dunphy WG (1995) Cell cycle regulation of a Xenopus Wee1-like kinase. Mol Biol Cell 6:119–134
55. Dunphy WG, Kumagai A (1991) The cdc25 protein contains an intrinsic phosphatase activity. Cell 67:189–196
56. Kumagai A, Dunphy WG (1991) The cdc25 protein controls tyrosine dephosphorylation of the cdc2 protein in a cell-free system. Cell 64:903–914
57. Blomberg I, Hoffmann I (1999) Ectopic expression of Cdc25A accelerates the G(1)/S transition and leads to premature activation of cyclin E- and cyclin A-dependent kinases. Mol Cell Biol 19:6183–6194
58. Hoffmann I, Draetta G, Karsenti E (1994) Activation of the phosphatase activity of human cdc25A by a cdk2-cyclin E dependent phosphorylation at the G1/S transition. EMBO J 13:4302–4310
59. Jinno S, Suto K, Nagata A, Igarashi M, Kanaoka Y, Nojima H et al (1994) Cdc25A is a novel phosphatase functioning early in the cell cycle. EMBO J 13:1549–1556
60. Lindqvist A, Kallstrom H, Lundgren A, Barsoum E, Rosenthal CK (2005) Cdc25B cooperates with Cdc25A to induce mitosis but has a unique role in activating cyclin B1-Cdk1 at the centrosome. J Cell Biol 171:35–45
61. Mailand N, Podtelejnikov AV, Groth A, Mann M, Bartek J, Lukas J (2002) Regulation of G(2)/M events by Cdc25A through phosphorylation-dependent modulation of its stability. EMBO J 21:5911–5920
62. Boutros R, Dozier C, Ducommun B (2006) The when and wheres of CDC25 phosphatases. Curr Opin Cell Biol 18:185–191
63. Gabrielli BG, De Souza CP, Tonks ID, Clark JM, Hayward NK, Ellem KA (1996) Cytoplasmic accumulation of cdc25B phosphatase in mitosis triggers centrosomal microtubule nucleation in HeLa cells. J Cell Sci 109(Pt 5):1081–1093
64. De Souza CP, Ellem KA, Gabrielli BG (2000) Centrosomal and cytoplasmic Cdc2/cyclin B1 activation precedes nuclear mitotic events. Exp Cell Res 257:11–21
65. Gabrielli BG, Clark JM, McCormack AK, Ellem KA (1997) Hyperphosphorylation of the N-terminal domain of Cdc25 regulates activity toward cyclin B1/Cdc2 but not cyclin A/Cdk2. J Biol Chem 272:28607–28614
66. Turowski P, Franckhauser C, Morris MC, Vaglio P, Fernandez A, Lamb NJ (2003) Functional cdc25C dual-specificity phosphatase is required for S-phase entry in human cells. Mol Biol Cell 14:2984–2998
67. Garner-Hamrick PA, Fisher C (1998) Antisense phosphorothioate oligonucleotides specifically down-regulate cdc25B causing S-phase delay and persistent antiproliferative effects. Int J Cancer 76:720–728
68. Vandre DD, Wills VL (1992) Inhibition of mitosis by okadaic acid: possible involvement of a protein phosphatase 2A in the transition from metaphase to anaphase. J Cell Sci 101(Pt 1):79–91
69. Skoufias DA, Indorato RL, Lacroix F, Panopoulos A, Margolis RL (2007) Mitosis persists in the absence of

Cdk1 activity when proteolysis or protein phosphatase activity is suppressed. J Cell Biol 179:671–685
70. Burgess A, Vigneron S, Brioudes E, Labbe JC, Lorca T, Castro A (2010) Loss of human Greatwall results in G2 arrest and multiple mitotic defects due to deregulation of the cyclin B-Cdc2/PP2A balance. Proc Natl Acad Sci USA 107:12564–12569
71. Castilho PV, Williams BC, Mochida S, Zhao Y, Goldberg ML (2009) The M phase kinase Greatwall (Gwl) promotes inactivation of PP2A/B55delta, a phosphatase directed against CDK phosphosites. Mol Biol Cell 20:4777–4789
72. Vigneron S, Brioudes E, Burgess A, Labbe JC, Lorca T, Castro A (2009) Greatwall maintains mitosis through regulation of PP2A. EMBO J 28:2786–2793
73. Zhao Y, Haccard O, Wang R, Yu J, Kuang J, Jessus C et al (2008) Roles of Greatwall kinase in the regulation of cdc25 phosphatase. Mol Biol Cell 19:1317–1327
74. Lorca T, Bernis C, Vigneron S, Burgess A, Brioudes E, Labbe JC et al (2009) Constant regulation of both the MPF amplification loop and the Greatwall-PP2A pathway is required for metaphase II arrest and correct entry into the first embryonic cell cycle. J Cell Sci 123:2281–2291
75. Gharbi-Ayachi A, Labbe JC, Burgess A, Vigneron S, Strub JM, Brioudes E et al (2010) The substrate of Greatwall kinase, Arpp19, controls mitosis by inhibiting protein phosphatase 2A. Science 330:1673–1677
76. Mochida S, Maslen SL, Skehel M, Hunt T (2010) Greatwall phosphorylates an inhibitor of protein phosphatase 2A that is essential for mitosis. Science 330:1670–1673
77. Nasmyth K, Haering CH (2009) Cohesin: its roles and mechanisms. Annu Rev Genet 43:525–558
78. Hauf S, Roitinger E, Koch B, Dittrich CM, Mechtler K, Peters JM (2005) Dissociation of cohesin from chromosome arms and loss of arm cohesion during early mitosis depends on phosphorylation of SA2. PLoS Biol 3:e69
79. Kitajima TS, Kawashima SA, Watanabe Y (2004) The conserved kinetochore protein shugoshin protects centromeric cohesion during meiosis. Nature 427:510–517
80. Llano E, Gomez R, Gutierrez-Caballero C, Herran Y, Sanchez-Martin M, Vazquez-Quinones L et al (2008) Shugoshin-2 is essential for the completion of meiosis but not for mitotic cell division in mice. Genes Dev 22:2400–2413
81. McGuinness BE, Hirota T, Kudo NR, Peters JM, Nasmyth K (2005) Shugoshin prevents dissociation of cohesin from centromeres during mitosis in vertebrate cells. PLoS Biol 3:e86
82. Salic A, Waters JC, Mitchison TJ (2004) Vertebrate shugoshin links sister centromere cohesion and kinetochore microtubule stability in mitosis. Cell 118:567–578
83. Kitajima TS, Sakuno T, Ishiguro K, Iemura S, Natsume T, Kawashima SA et al (2006) Shugoshin collaborates with protein phosphatase 2A to protect cohesin. Nature 441:46–52
84. Riedel CG, Katis VL, Katou Y, Mori S, Itoh T, Helmhart W et al (2006) Protein phosphatase 2A protects centromeric sister chromatid cohesion during meiosis I. Nature 441:53–61
85. Tang Z, Shu H, Qi W, Mahmood NA, Mumby MC, Yu H (2006) PP2A is required for centromeric localization of Sgo1 and proper chromosome segregation. Dev Cell 10:575–585
86. Wittmann T, Hyman A, Desai A (2001) The spindle: a dynamic assembly of microtubules and motors. Nat Cell Biol 3:E28–E34
87. Helps NR, Luo X, Barker HM, Cohen PT (2000) NIMA-related kinase 2 (Nek2), a cell-cycle-regulated protein kinase localized to centrosomes, is complexed to protein phosphatase 1. Biochem J 349:509–518
88. Meraldi P, Nigg EA (2001) Centrosome cohesion is regulated by a balance of kinase and phosphatase activities. J Cell Sci 114:3749–3757
89. Mi J, Guo C, Brautigan DL, Larner JM (2007) Protein phosphatase-1alpha regulates centrosome splitting through Nek2. Cancer Res 67:1082–1089
90. Sumara I, Gimenez-Abian JF, Gerlich D, Hirota T, Kraft C, de la Torre C et al (2004) Roles of polo-like kinase 1 in the assembly of functional mitotic spindles. Curr Biol 14:1712–1722
91. Girdler F, Gascoigne KE, Eyers PA, Hartmuth S, Crafter C, Foote KM et al (2006) Validating Aurora B as an anti-cancer drug target. J Cell Sci 119:3664–3675
92. Bayliss R, Sardon T, Vernos I, Conti E (2003) Structural basis of Aurora-A activation by TPX2 at the mitotic spindle. Mol Cell 12:851–862
93. Sessa F, Mapelli M, Ciferri C, Tarricone C, Areces LB, Schneider TR et al (2005) Mechanism of Aurora B activation by INCENP and inhibition by hesperadin. Mol Cell 18:379–391
94. Huse M, Kuriyan J (2002) The conformational plasticity of protein kinases. Cell 109:275–282
95. Zeng K, Bastos RN, Barr FA, Gruneberg U (2010) Protein phosphatase 6 regulates mitotic spindle formation by controlling the T-loop phosphorylation state of Aurora A bound to its activator TPX2. J Cell Biol 191:1315–1332
96. Ruchaud S, Carmena M, Earnshaw WC (2007) Chromosomal passengers: conducting cell division. Nat Rev Mol Cell Biol 8:798–812
97. Posch M, Khoudoli GA, Swift S, King EM, Deluca JG, Swedlow JR (2010) Sds22 regulates aurora B activity and microtubule-kinetochore interactions at mitosis. J Cell Biol 191:61–74
98. Sugiyama K, Sugiura K, Hara T, Sugimoto K, Shima H, Honda K et al (2002) Aurora-B associated protein phosphatases as negative regulators of kinase activation. Oncogene 21:3103–3111

99. Liu D, Vleugel M, Backer CB, Hori T, Fukagawa T, Cheeseman IM et al (2010) Regulated targeting of protein phosphatase 1 to the outer kinetochore by KNL1 opposes Aurora B kinase. J Cell Biol 188:809–820
100. Wan X, O'Quinn RP, Pierce HL, Joglekar AP, Gall WE, DeLuca JG et al (2009) Protein architecture of the human kinetochore microtubule attachment site. Cell 137:672–684
101. Welburn JP, Vleugel M, Liu D, Yates JR 3rd, Lampson MA, Fukagawa T et al (2010) Aurora B phosphorylates spatially distinct targets to differentially regulate the kinetochore-microtubule interface. Mol Cell 38:383–392
102. Musacchio A, Salmon ED (2007) The spindle-assembly checkpoint in space and time. Nat Rev Mol Cell Biol 8:379–393
103. Stegmeier F, Amon A (2004) Closing mitosis: the functions of the Cdc14 phosphatase and its regulation. Annu Rev Genet 38:203–232
104. Mocciaro A, Schiebel E (2010) Cdc14: a highly conserved family of phosphatases with non-conserved functions? J Cell Sci 123:2867–2876
105. Mocciaro A, Berdougo E, Zeng K, Black E, Vagnarelli P, Earnshaw W et al (2010) Vertebrate cells genetically deficient for Cdc14A or Cdc14B retain DNA damage checkpoint proficiency but are impaired in DNA repair. J Cell Biol 189:631–639
106. Ferrigno P, Langan TA, Cohen P (1993) Protein phosphatase 2A1 is the major enzyme in vertebrate cell extracts that dephosphorylates several physiological substrates for cyclin-dependent protein kinases. Mol Biol Cell 4:669–677
107. Mayer-Jaekel RE, Ohkura H, Ferrigno P, Andjelkovic N, Shiomi K, Uemura T et al (1994) Drosophila mutants in the 55 kDa regulatory subunit of protein phosphatase 2A show strongly reduced ability to dephosphorylate substrates of p34cdc2. J Cell Sci 107(Pt 9):2609–2616
108. Mayer-Jaekel RE, Ohkura H, Gomes R, Sunkel CE, Baumgartner S, Hemmings BA et al (1993) The 55 kd regulatory subunit of Drosophila protein phosphatase 2A is required for anaphase. Cell 72:621–633
109. Mochida S, Ikeo S, Gannon J, Hunt T (2009) Regulated activity of PP2A-B55 delta is crucial for controlling entry into and exit from mitosis in Xenopus egg extracts. EMBO J 28:2777–2785
110. Schmitz MH, Held M, Janssens V, Hutchins JR, Hudecz O, Ivanova E et al (2010) Live-cell imaging RNAi screen identifies PP2A-B55alpha and importin-beta1 as key mitotic exit regulators in human cells. Nat Cell Biol 12:886–893
111. Axton JM, Dombradi V, Cohen PT, Glover DM (1990) One of the protein phosphatase 1 isoenzymes in Drosophila is essential for mitosis. Cell 63:33–46
112. Chen F, Archambault V, Kar A, Lio P, D'Avino PP, Sinka R et al (2007) Multiple protein phosphatases are required for mitosis in Drosophila. Curr Biol 17:293–303
113. Wu JQ, Guo JY, Tang W, Yang CS, Freel CD, Chen C et al (2009) PP1-mediated dephosphorylation of phosphoproteins at mitotic exit is controlled by inhibitor-1 and PP1 phosphorylation. Nat Cell Biol 11:644–651
114. Trinkle-Mulcahy L, Andersen J, Lam YW, Moorhead G, Mann M, Lamond AI (2006) Repo-Man recruits PP1 gamma to chromatin and is essential for cell viability. J Cell Biol 172:679–692
115. Vagnarelli P, Hudson DF, Ribeiro SA, Trinkle-Mulcahy L, Spence JM, Lai F et al (2006) Condensin and Repo-Man-PP1 co-operate in the regulation of chromosome architecture during mitosis. Nat Cell Biol 8:1133–1142
116. Qian J, Lesage B, Beullens M, Van Eynde A, Bollen M (2011) PP1/Repo-man dephosphorylates mitotic histone H3 at T3 and regulates chromosomal aurora B targeting. Curr Biol 21:766–773
117. Kelly AE, Ghenoiu C, Xue JZ, Zierhut C, Kimura H, Funabiki H (2010) Survivin reads phosphorylated histone H3 threonine 3 to activate the mitotic kinase Aurora B. Science 330:235–239
118. Wang F, Dai J, Daum JR, Niedzialkowska E, Banerjee B, Stukenberg PT et al (2010) Histone H3 Thr-3 phosphorylation by Haspin positions Aurora B at centromeres in mitosis. Science 330:231–235
119. Guttinger S, Laurell E, Kutay U (2009) Orchestrating nuclear envelope disassembly and reassembly during mitosis. Nat Rev Mol Cell Biol 10:178–191
120. Thompson LJ, Bollen M, Fields AP (1997) Identification of protein phosphatase 1 as a mitotic lamin phosphatase. J Biol Chem 272:29693–29697
121. Steen RL, Martins SB, Tasken K, Collas P (2000) Recruitment of protein phosphatase 1 to the nuclear envelope by A-kinase anchoring protein AKAP149 is a prerequisite for nuclear lamina assembly. J Cell Biol 150:1251–1262
122. Landsverk HB, Kirkhus M, Bollen M, Kuntziger T, Collas P (2005) PNUTS enhances in vitro chromosome decondensation in a PP1-dependent manner. Biochem J 390:709–717
123. Wei JH, Seemann J (2009) Mitotic division of the mammalian Golgi apparatus. Semin Cell Dev Biol 20:810–816
124. Lowe M, Gonatas NK, Warren G (2000) The mitotic phosphorylation cycle of the cis-Golgi matrix protein GM130. J Cell Biol 149:341–356
125. Brautigan DL (2013) Protein Ser/Thr phosphatases–the ugly ducklings of cell signalling. FEBS J 280:324–345
126. Gingras AC, Caballero M, Zarske M, Sanchez A, Hazbun TR, Fields S et al (2005) A novel, evolutionarily conserved protein phosphatase complex involved in cisplatin sensitivity. Mol Cell Proteomics 4:1725–1740
127. Glatter T, Wepf A, Aebersold R, Gstaiger M (2009) An integrated workflow for charting the human interaction proteome: insights into the PP2A system. Mol Syst Biol 5:237
128. Shinde SR, Maddika S (2016) PTEN modulates EGFR late endocytic trafficking and degradation by dephosphorylating Rab7. Nat Commun 7:10689

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