Protein phosphatases and their regulation in the control of mitosis

Satoru Mochida[1+] and Tim Hunt[2]

Address 1; Honjo-Kyoyotou 206, Kumamoto University, 2-2-1 Honjo, Kumamoto city, Kumamoto 860-0811, JAPAN

Address 2; Cancer Research UK, Clare Hall Laboratories, South Mimms, Herts EN6 3LD, UK

[+]Corresponding author

E-mail; mochida@kumamoto-u.ac.jp

Tel; +81 373 6819

Fax; +81 373 6819

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Abstract

Cell cycle transitions depend on protein phosphorylation and dephosphorylation. The discovery of cyclin dependent kinases (CDKs) and their mode of activation by their cyclin partners explained many important aspects of cell cycle control. As the cell cycle is basically a series of recurrences of a defined set of events, protein phosphatases must obviously be as important as kinases. However, our knowledge about phosphatases lags well behind that of kinases. We still do not know exactly which phosphatase(s) is/are truly responsible for dephosphorylating CDK substrates, and we know very little about whether and how protein phosphatases are regulated. Here, we summarise our present understanding of the phosphatases that are important in the control of the cell cycle and pose the questions that need to be answered as regards the regulation of protein phosphatases.

Background

After the discovery of CDK-cyclin complexes as main regulators of the cell cycle (Meijer et al, 1989; Simanis & Nurse, 1986), various kinases have been identified for their specialized functions for mitosis (Table 1). Dynamic change of their activity and localization and identification of functional substrates significantly promoted our understanding about how visible mitotic events, such as nuclear envelope breakdown, chromosome condensation/cohesion, spindle assembly and so on, are controlled by the reversible chemical reaction, protein phosphorylation. As is easily imagined, once a phospho-dependent event is complete, dephosphorylation is required for cells to return into the basal state for the next round of the cell cycle. Protein phosphatases are

able to hydrolyze the phosphoesters of serine, threonine and/or tyrosine residues, thereby erasing the kinases' marks. In this sense, it is phosphatases that are the main contributor for ending mitosis. But in fact, as we shall see below, protein phosphatases also play important roles before and during mitosis. Finally, it is crucial in all cases to achieve good coordination of phosphatases with opposing kinases, that is, the control of phosphatases, a topic that has received little attention up to now (Sidebar A).

Avoid futile cycles!

We would expect to find mechanisms to avoid the futile cycles that would occur if kinases and their counteracting phosphatases were simultaneously active (Fig 1 and Sidebar A). This applies especially to proteins that undergo almost quantitative conversion from an unphosphorylated state to a heavily phosphorylated state, such as APC3 (Cdc27) undergoes as cells enter mitosis (see Figure 2A). It is evident that phosphatases are active at the end of mitosis to restore the phosphorylation state of such proteins to their interphase state of hypophosphorylation, and equally clear that one or more kinases are activated at the onset of mitosis to bring about the mitotic hyperphosphorylated state. What one cannot tell from simply looking at the fractional phosphorylation, however, is the extent to which this interconversion necessarily entails reciprocal inhibition of phosphatases as the kinases are activated, and activation of phosphatases when the kinase activity is diminished.

Spatial regulation of phosphatases

Some phosphatases have recently been found to be regulated by their intracellular localization (Sidebar A). For example, Cdc14 in budding yeast is sequestered in the

nucleolus until metaphase, then released into whole nucleus and cytoplasm by the FEAR and MEN systems (Table 1) (Shou et al, 1999), mainly to dephosphorylate CDK substrates (Visintin et al, 1998). Another good example is the PP2A-B56/Shugoshin complex, which localises to pericentromeric region where it keeps cohesin complexes dephosphorylated. Cohesin complexes on chromosome arm region are phosphorylated by several kinases and thereby removed from DNA well before the metaphase-anaphase transition (Table 1). The dephosphorylated population of cohesin at the pericentromeric region are sufficient to keep sister chromatids attached and allow chromosome separation, coordinated with CDK inactivation, in anaphase (Hauf et al, 2005; Ishiguro et al, 2010; Katis et al, 2010; Kitajima et al, 2006). PP4 is also regulated by its localization. During interphase, some population of PP4 localizes at centrosome and suppresses unscheduled CDK1 activation. On entering mitosis, PP4 is dispersed into cytoplasm, thereby allows its substrate NDEL1, a protein important for microtubule organization, to be phosphorylated by CDK1 (Toyo-oka et al, 2008).

Activity-level regulation of phosphatases

Another stream in phosphatase research is the discoveries of regulation at their enzymatic activity level (Sidebar A). Thus, PP1 has a highly conserved CDK target motif at its C terminus, and phosphorylation of this site decreases its phosphatase activity *in vitro* (Yamano et al, 1994). In addition, inhibitor-1 of PP1 is phosphorylated and activated during mitosis (Wu et al, 2009). These data support the idea that there is a reduction in PP1 activity in mitosis, although the actual change of PP1 activity *in vivo* is still unknown. Another example is the activation of calcium/calmodulin-activated phosphatase, PP2B/calcineurin upon exit from meiotic

M phase-II, brought about by the fertilization-induced calcium ion flux. This activation is important for proper cyclin degradation and timely entry into the embryonic cell cycle (Mochida & Hunt, 2007; Nishiyama et al, 2007). The mechanism of calcineurin activation had been well studied (Klee et al, 1998). We identified a phosphatase activity, which is high during interphase and very low in mitosis. This turned out to be a particular form of PP2A, PP2A-B55δ (Fig 2A) (Mochida et al, 2009). But before going into details of the regulatory mechanism of this phosphatase, we would like to make a note about the use of phosphatase inhibitors in the study of the control of mitosis.

Avoid an abusive interpretation of useful inhibitors

When analysing and identifying phosphatases, it is important to understand and define the variety of protein phosphatase holocomplexes. For example, the active form of PP2A is a hetero-trimer, composed of catalytic (C), scaffolding (A) and regulatory (B) subunits. In humans, there are 2 Cs (α & β), 2 As (α & β) and nearly 20 different Bs belonging to four different families (the B55/B, B56/B', B" and B"' sub-families) (Eichhorn et al, 2009), suggesting that nearly 80 different PP2A holo-complexes could exist in a cell. All too often, researchers simply refer to "PP2A" without specifying the flavour. This variety applies equally to other PPP family members, including PP1, PP4 and PP6 (Ceulemans & Bollen, 2004; Gingras et al, 2005; Hastie et al, 2000; Kloeker & Wadzinski, 1999; Luke et al, 1996; Stefansson et al, 2008). As each of the holocomplexes are likely to have specific functions *in vivo*, it is essential to analyse them one by one. These considerations mean that often-used tools, phosphatase inhibitors like okadaic acid and microcystin, are apt to give misleading results. First, these inhibitors do not distinguish among different holo-complexes

containing identical catalytic subunits. Second, different phosphatases are expressed *in vivo* at various concentrations (sub nM to low μM level), indicating that the IC50 values of these inhibitors are an unreliable guide to identify phosphatases that are inhibited *in vivo* at a certain concentration of inhibitor. Those IC50s were originally calculated *in vitro,* using very diluted solutions of protein phosphatases (Takai et al, 1987; Yoshizawa et al, 1990). Third, some phosphatases show rather similar sensitivities to these inhibitors. For example, the sensitivities of PP2A and PP4 (and very likely PP6) for okadaic acid are around 0.1 to 0.3 nM, too close to distinguish from each other. Finally, we do not know how much inhibitor passes through the cell membrane. For all of these reasons, interpretation of data using these inhibitors is necessarily limited and any results should be confirmed using other methods, such as gene knock-out. To further analyse specific functions of a particular phosphatase complex, it is absolutely necessary to identify the appropriate regulatory subunit. Of course, these inhibitors can still provide useful clues if they are used carefully.

Is PP2A-B55δ the phosphatase for CDK substrates?

Our attention was drawn to the question of which phosphatase(s) were responsible for mitotic exit by the discovery that PP2B/calcineurin was activated when crude *Xenopus* egg extracts are released from CSF-arrest (arrested at meiotic metaphase-II with high CDK activity - by the combination of Mos kinase and Erp1/Emi2) by the addition of $CaCl_2$ (Mochida & Hunt, 2007; Nishiyama et al, 2007). It turned out that inhibition of PP2B (by cyclosporin A) seriously delayed the return to interphase in this setting, and we designed a substrate that could monitor phosphatase activity in these crude extracts. To our surprise we found that the main role of PP2B/calcineurin was to allow the activation of a second phosphatase, not itself calcium activated, that

was normally highly active in interphase and inhibited in meiosis and mitosis (Fig 2A) (Mochida & Hunt, 2007). This regulation of the phosphatase activity was abolished after the addition of buffer to the concentrated egg extracts, so we had to use an immunodepletion technique, instead of standard biochemical fractionation/purification methods, to identify this fluctuating phosphatase activity. It proved to be a particular form of PP2A that contained a B55δ regulatory subunit (Mochida et al, 2009). In extracts that had been depleted of PP2A-B55δ, mitotic phosphorylation was accelerated at a lower-than-usual concentration of cyclin B (Mochida et al, 2009). Furthermore, histone1 kinase activity, which reflects the level of Cdc2 kinase, was enhanced when PP2A-B55δ was depleted in interphase egg extracts. This is reminiscent of the experiments leading to the characterization of INH (Solomon et al, 1990). INH was originally defined as an activity that inhibited the activation of MPF in *Xenopus* oocytes, and was later identified as a form of PP2A (Cyert & Kirschner, 1988; Lee et al, 1991). We found that depletion of PP2A-B55δ led to a failure to dephosphorylate mitotic CDK substrates at the end of mitosis, although cyclin degradation and CDK inactivation took place more or less normally (Mochida et al, 2009). These observations initially suggested that PP2A-B55δ was the main phosphatase for CDK substrates. However, when B55δ was depleted after the egg extracts entered mitosis, it was no longer required to exit mitosis (Mochida et al, 2009). This puzzling result suggested that although PP2A-B55δ is required for mitotic exit, its critical role for mitotic exit is already complete before entering mitosis. We have no idea how PP2A-B55δ affects mitotic exit in the preceding interphase, nor how many CDK substrates are dephosphorylated by PP2A-B55δ. In any case, PP2A-B55δ is clearly not the only phosphatase that dephosphorylates CDK substrates. There must be other phosphatases acting at mitotic exit. For example, a form of PP1 is a

very good candidate for this role, as a considerable body of data has already implicated it in mitotic exit. Cdc14 could be another candidate, although the function(s) of Cdc14 homologues in higher eukaryotes remain unclear (Mocciaro & Schiebel, 2010; Taylor et al, 1997; Visintin et al, 1998).

Greatwall kinase regulates PP2A-B55δ activity in mitosis

The Greatwall (Gwl) gene was originally identified as the *Scant* (Scott of the Antarctic) mutation in *Drosophila* (White-Cooper et al, 1996). *Scant* later turned out to encode a protein kinase that is important for mitosis (Yu et al, 2004). *Drosophila* mutants deficient in Gwl showed defects in chromosome condensation and delayed cell cycle progression throughout late G2 phase to mitosis. The kinase activity of Gwl increases as cells enter mitosis, during which Gwl itself is highly phosphorylated, at least in part by CDK1. Further analysis using *Xenopus* egg extracts revealed that Gwl is not only important for entering mitosis, but was also required for maintaining the CSF-arrested mitotic state (Yu et al, 2006). If Gwl is depleted from mitotic egg extracts (CSF), active CDK1 is inactivated by inhibitory phosphorylation on its Tyr15 residue, rather than by cyclin proteolysis (Yu et al, 2006). These findings suggest that the role of Gwl in mitosis is to control the CDK1 regulators Cdc25 and/or Wee1, which are themselves substrates of CDK1 (Hoffmann et al, 1993; Izumi & Maller, 1993; Mueller et al, 1995). Strikingly, even in the presence of high CDK1 activity, loss of Gwl induces dephosphorylation of mitotic phosphoproteins, strongly suggesting that Gwl acts as an inhibitor of the protein phosphatase(s) that antagonise CDK1. Indeed, PP2A-B55δ is activated upon depletion of Gwl from CSF-arrested mitotic extracts (Castilho et al, 2009; Vigneron et al, 2009). Oddly, however, Gwl

does not phosphorylate any subunit of this phosphatase complex, so its mode of action was obscure for some time.

Ensa/ARPP-19, the first Gwl substrates, inhibit PP2A-B55

Two small, heat-stable proteins called alpha-endosulfine (Ensa) and ARPP-19 share almost 70% sequence identity and are members of a highly conserved protein family (Fig 2B). ARPP-19 and its short form ARPP-16 were first identified as major substrates for protein kinase A in brain (Girault et al, 1990; Horiuchi et al, 1990). Therefore, they appeared to be involved in dopamine signalling in the post-synaptic neuron, where signalling cascades using protein phosphorylation are important (Dulubova et al, 2001). Ensa was initially thought to be an endogenous ligand for the sulfonylurea receptor and was supposed to be involved in the control of insulin secretion (Virsolvy-Vergine et al, 1992). But this original idea could not be confirmed and now seems unlikely, because of the absence of a secretion-signal sequence in Ensa. And very little Ensa was found in biological membrane fractions (Gros et al, 2002). Thus, for nearly 20 years after the identification of these proteins, no molecular function had been found. The first evidence for the importance of Ensa in cell cycle control came from a study in *Drosophila*, which has only one gene in this protein family. An RNAi screening using somatic S2 cells identified Endos (the name for Ensa in *Drosophila*) as a protein important for mitotic chromosome alignment and normal spindle length (Goshima et al, 2007). *Drosophila* oocytes deficient in Endos show high CDK activity with low phosphorylation of CDK substrates, indicating that lack of Ensa somehow changes the balance between kinase and phosphatase (Von Stetina et al, 2008). We and others independently discovered that Ensa and ARPP-19 are phosphorylated by Gwl at a highly conserved Ser residue —S67 in *Xenopus* Ensa

(Fig 2B)— becoming potent inhibitors of PP2A-B55δ (Fig 2C) (Gharbi-Ayachi et al, 2010; Mochida et al, 2010). Importantly, this inhibition is highly specific for PP2A-B55δ; other forms of PP2A are unaffected (Mochida et al, 2010). Although the exact sequence that is phosphorylated by Gwl (KYFDSGDYNM) is found only in these two small proteins, Gwl could have other substrates, as the stringency of Gwl substrate recognition sequence is unknown.

Reversing the balance of CDK1 and PP2A-B55δ

Phosphorylation of Ensa/ARPP-19 by Gwl is essential for CDK substrates to be highly phosphorylated in *Xenopus* embryonic mitosis. In cycling egg extracts that lack Ensa, although Tyr15 dephosphorylation and full CDK activation occur to the same level as in control extracts (albeit somewhat delayed) (Mochida et al, 2010), PP2A-B55δ activity is not suppressed and CDK substrates are never fully phosphorylated. A 3-fold increase in PP2A-B55δ concentration induces similar phenotype, presumably because the increased PP2A titrates out endogenous Ensa (Mochida et al, 2009). Furthermore, the addition of active (thiophosphorylated) Ensa is enough to induce significant phosphorylation of CDK substrates even at very low levels of cyclin, below those required for normal mitosis. These observations collectively suggest that even full CDK1 activation is unable to promote entry into mitosis; inactivation of phosphatase(s) is also required. The Gwl pathway evolved to achieve this seesaw-like relationship.

A word of caution is necessary, however, because not all cell divisions seem to depend on the Gwl-Ensa/ARPP-19 system. For example, although *Drosophila* that lack Endos are inviable, loss of one copy of the twins/aar gene that encodes the B55 subunit of PP2A rescues the lethality (although not the female sterility) (Rangone et al,

2011). It is difficult to interpret these data with our current understanding of the pathway.

Specificity and regulation of Ensa/ARPP-19 family

Unlike okadaic acid, Ensa is highly specific for the particular species of PP2A that contains the B55δ subunit (Fig 2C) and does not bind other forms of PP2A, containing B56ε, B56γ or B"/PR48 regulatory subunits (Mochida et al, 2010). Thus, different PP2A holocomplexes are distinctively regulated. It is highly probable, however, that other isoforms of B55 (α & β) are also targeted by Ensa/ARPP-19 (Manchado et al, 2010; Schmitz et al, 2010).

In addition to the Gwl phosphorylation site, Ensa/ARPP-19 family proteins have another highly conserved phosphorylation site at their C terminus (Girault et al, 1988). This site —Ser109 in *Xenopus* Ensa— seems to be phosphorylated by protein kinases that prefer basic residues preceding the phosphorylation site, such as PKA and Chk1 (Fig 1B). *Xenopus* Ensa and ARPP-19 have one more possible phosphorylation site in their N-terminal region, Thr 28 (Fig 1B), which matches the CDK consensus (S/T-P-X-K/R, where X can be any amino acid) and can be phosphorylated by CDK2 *in vitro* (Mochida et al, 2010). The functions of these additional phosphorylation sites are of great interest and it is important to know when they are phosphorylated *in vivo*. Multiple phosphorylation sites in such small phosphatase inhibitor remind us of PP1 inhibitor proteins, such as DARPP-32 and Inhibitor-1 (Hemmings et al, 1984; Huang & Glinsmann, 1976; Oliver & Shenolikar, 1998). Like them, the Ensa/ARPP-19 family could be an integrator of multiple signals.

Alternative functions of Gwl/Ensa/ARPP-19

In *Xenopus* and *Drosophila* (and probably also in human cells), Gwl and Ensa/ARPP-19 family proteins are clearly involved in mitotic regulation (Gharbi-Ayachi et al, 2010; Mochida et al, 2010). In budding yeast, however, the Rim15 kinase (the closest homologue of Gwl) and its substrates Igo1 and Igo2 (homologues of Ensa and ARPP-19), are important for the response to nutritional deprivation under the control of TOR (Talarek et al, 2010). The phosphatase targeted by Igo1 and Igo2 in yeast remains to be identified, but Rim15 phosphorylates Igo1 and 2 at the same sites as Gwl does in *Xenopus* (Fig 2B). It would be important to check if Igo1 and Igo2 inhibit PP2A-Cdc55 —the budding yeast homologue of the B55 family (Healy et al, 1991)—, and to characterize the substrates of this phosphatase and discover what kinase phosphorylates these sites (Sidebar A). If this kinase is activated by a TOR signal (or by TOR itself), a picture analogous to cell cycle control would emerge in a different context of biological function. That is, the Rim15 pathway might act by changing the balance of a paired protein kinase and phosphatase in the context of the response to starvation.

It should be noted that ARPP-19 was first identified in the brain, where many signals are rapidly changing (Horiuchi et al, 1990; Walaas et al, 2011). The balance of protein kinase and phosphatase could be changed rapidly and coordinately by using the MAST-L kinase —the Gwl homologue in humans— and ARPP-19 (Burgess et al, 2010; Voets & Wolthuis, 2010). For example, CDK5 could be a candidate for its antagonizing kinase and tau protein for its substrate in this context (Sidebar A) (Hellmich et al, 1992).

Key factors for the suddenness of mitotic entry

Wee1 is the main kinase that phosphorylates the Tyr15 residue of CDK1, the dephosphorylation of which by the Cdc25 phosphatase is essential for the activation of CDK1. As shown in Figure 3, Cdc25 is activated, whereas Wee1 is turned off by phosphorylation brought about by CDK1. Thus, Cdc25 and Wee1 form positive and negative feedback loops, respectively, with CDK1 (Hoffmann et al, 1993; Izumi & Maller, 1993; Mueller et al, 1995). As we originally identified PP2A-B55δ as a phosphatase able to act on CDK substrates, Cdc25 and Wee1 could be two physiological targets of PP2A-B55δ (Mochida et al, 2009). If this is the case, then PP2A-B55δ contributes to the suppression of premature CDK activation by maintaining these two major CDK regulators in their hypophosphorylated state (Fig 3). This model raises the question of how and by what triggers the transition from interphase to mitosis is triggered. Given that the balance between CDK1 and its phosphatases is the target of this unknown triggering mechanism, protein phosphatases that dephosphorylate Gwl and Ensa/ARPP-19 during interphase must be important —they are labelled PPase-X and PPase-Y in Fig 3 (Sidebar A). PP1 is very likely to be involved in the reversal of either Gwl or Ensa/ARPP-19, or both in addition to the multiple roles of different PP1 complexes in mitosis (Wurzenberger & Gerlich, 2011). When the balance between kinase and phosphatase for Gwl and/or Ensa/ARPP-19 is changed, the Gwl pathway would get fired to induce rapid mitotic phosphorylation. Thus, the activating and deactivating mechanisms of Gwl and Ensa/ARPP-19 are extremely important not only for the occurrence of, but also for the kinetics of mitotic entry. Present evidence indicates that CDK is essential, but not sufficient for Gwl activation. A report from the Montpellier group about AGC kinase activation (Vigneron et al, 2011) is probably not the last word on the subject. The observation that a small population of PP2A-B55 could be found associated with Gwl

in interphase, but not in mitosis, raises the possibility that PP2A-B55 itself is involved in keeping the Gwl pathway turned off in interphase (Yamamoto et al, 2011). The existence of all these positive and negative feedback loops is probably to be expected of a reversible flip-flop switch, but from a biological perspective we need to know whose finger is on the trigger, so to speak (Fig 3).

Conclusion and perspectives

Considering that CDK1 has hundreds of substrates and that a number of other protein kinases, such as Aurora A and B, Polo, Wee1 and Myt1 are involved in entry into mitosis and mitotic progression, a number of different protein phosphatases are probably involved in the reversal or regulation of these processes (Table 1). A systematic survey in *Drosophila* using RNAi implicated no fewer than 22 protein phosphatases, although PP1 and PP2A were prominent among them (Chen et al, 2007). Phosphatases in addition to kinases would contribute to the fine-tuning of cellular events. We obviously need to pay fresh attention to protein phosphatases, and refine our view of them.

Sidebar A: In need of answers

i) It would be crucial to identify the phosphatase(s) that dephosphorylate Gwl and Ensa/ARPP-19 to explain how the Gwl-Ensa/ARPP-19 pathway is switched off, or reset, for the next round of the cell cycle.

ii) It is also important to explore the role of the Gwl-Ensa/ARPP-19 system in different biological contexts —such as the mammalian nervous system— and in different organisms (yeast and nematodes compared to insects and humans).

iii) More generally speaking, as it becomes clear that protein phosphatases can be highly and specifically regulated, we need to elucidate the details of their control mechanisms, especially in terms of the balance with their partner kinases.

iv) How many other of the PPP family of phosphatases can be switched on and off? Only biochemistry will tell!

Glossary

AGC kinases, a family including PKA, PKG and PKC members

CDK, cyclin-dependent kinase

CSF, cytostatic factor

IC50, concentration that inhibits 50% of activity

MPF, Maturation-promoting factor

PP1, type-1 protein phosphatase

PP2A, type-2A protein phosphatase

Gwl, Greatwall kinase

ARPP-16/19, cyclic-AMP regulated phosphoprotein of 16/19 kDa

Ensa, alpha-Endosulfine

PKA, cyclic-AMP activated protein kinase

DARPP-32, dopamine and cAMP-regulated phosphoproteins of 32 kDa

TOR, target of rapamycin.

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Conflict of interest

The authors declare that they have no conflict of interest.

Figure legends

Figure 1, A protein kinase adds while a phosphatase removes phosphate residue on a substrates. If these mutually antagonistic enzymes work at the same time, it not only results in a waste of ATP, but full switchlike interconversion of the phosphorylation state of the substrate is impossible. For this to occur, the two enzymes should work alternately, ideally while communicating each other.

Figure 2, alpha-**Endosulfine and ARPP-19 are Greatwall-dependent inhibitors of PP2A-B55.** A) Schematic diagram of CDK1 and PP2A-B55δ activity during cell cycle is shown. The patterns of CDK1 and PP2A-B55δ activity are complementary to each other; CDK1 activity is shown in red and PP2A-B55 activity in green. The phosphorylation status of Apc3/Cdc27 reflects the ratio of kinase to phosphatase activity (upper bands indicate mitotic hyperphosphorylation). B) Sequence alignment of the Ensa subfamily from yeast to human. Three possible phosphorylation sites are indicated with arrows. The CDK consensus site is found only in *Xenopus* Ensa, but is well conserved in the ARPP-19 subfamily. C) Protein phosphatase assay using a model CDK substrate and a catalytic C monomer, A+C dimer or hetero-trimer holocomplex containing B55δ. Ensa phosphorylated by Gwl (red bars) inhibits PP2A trimeric holocomplexes that contain B55δ, but not dimeric or monomeric PP2A complexes. Figure 1C is a modified version of Figure 2A from Mochida et al., 2010.

Figure 3, **Factors that control the Greatwall pathway during the cell cycle**. Red arrows are those promoting mitosis, whereas blue ones support interphase. $+PO_4$ and $-PO_4$ denote phosphorylation and dephosphorylation, respectively. PPases-X and -Y are the as-yet-unidentified protein phosphatases that deactivate the Gwl pathway.

Table 1, **Examples of kinases, phosphatases and their substrates important for mitosis.** Number of each phopshosite shows its position in human (*1) or in *Xenopus* protein (*2). References: *3(Agostinis et al, 1992; Mayer-Jaekel et al, 1993; Mochida et al, 2009; Schmitz et al, 2010; Sola et al, 1991), *4(Stone et al, 1993; Wu et al, 2009), *5(Visintin et al, 1998), *6(Toyo-oka et al, 2008), *7(Mochida & Hunt, 2007; Nishiyama et al, 2007), *8(Margolis et al, 2006a), *9(Hauf et al, 2005; Ishiguro et al, 2010; Katis et al, 2010; Kitajima et al, 2006), *10(Margolis et al, 2006b; Peng et al, 1997), *11(Zeng et al, 2010), *12(Kim et al, 2010), *13(Qian et al, 2011), *14(Gharbi-Ayachi et al, 2010; Mochida et al, 2010), *15(Lundgren et al, 1991; Strausfeld et al, 1991)

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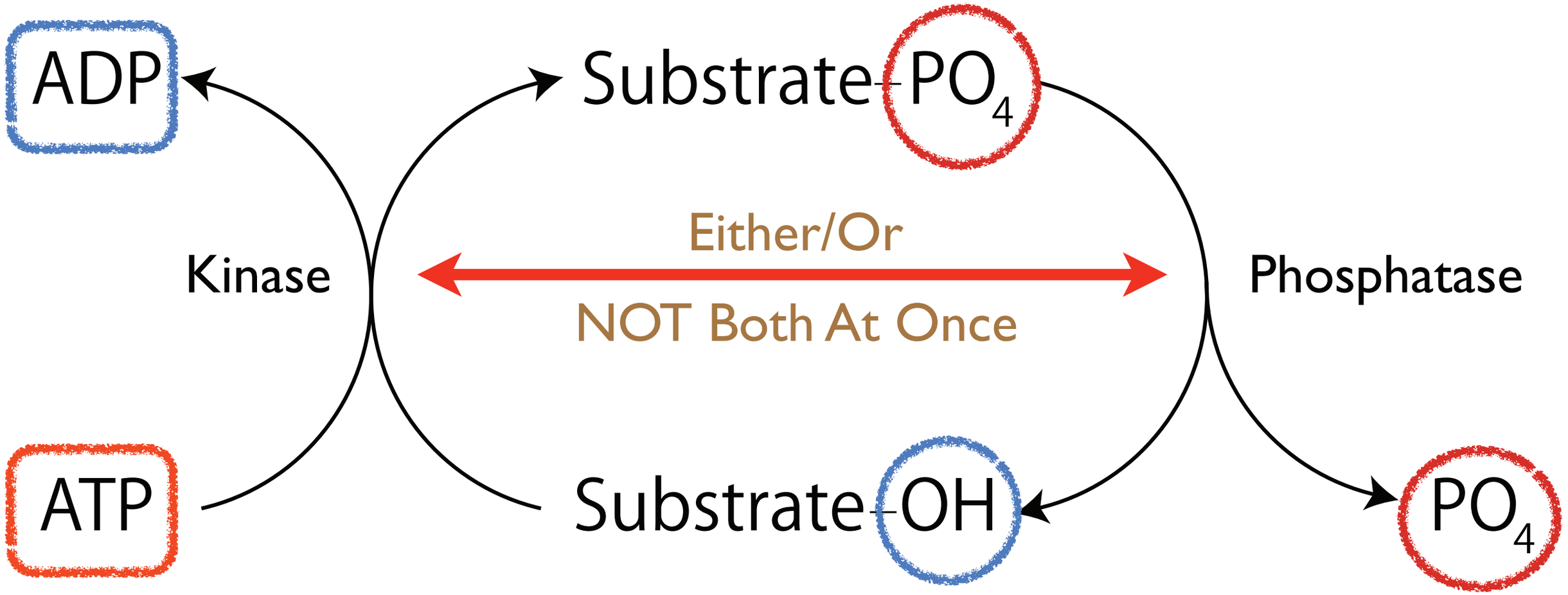
Kinase	Substrate (phosphosite)	Phosphatase	Reference
Cdk1-cyclin B	Broad range of Cdk1 substrates	PP2A-B55	*3
		PP1	*4
		Cdc14	*5
	NDEL1 (T219[*1])	PP4	*6
	Fizzy	PP2B/calcineurin	*7
p90Rsk	Erp1[Emi2]		
Cdk2-cyclin E	Cdc25 (T138[*2])	PP2A-B56	*8
Casein kianse 1	Rec8	PP2A-B56/Shugosin	*9
Polo-like kinase 1	Scc3/SA2		
Chk1	Cdc25 (S287[*2])	PP1	*10
Aurora A	Aurora A (T288 in T-loop[*1])	PP6-SAPs/ANKRDs	*11
Aurora B	CENP-E (T422[*1])	PP1	*12
Haspin	Histone H3 (T3)	PP1γ-Repo-man	*13
Greatwall	α-Endosulfine/ARPP-19 (S67[*2])	Unknown	*14
Wee1/Myt1	Cdk1 (T14/Y15)	Cdc25	*15

Table 1, Combinations of protein kinases, phosphatases and their substrates important for mitosis

Avoid Futile Cycles!



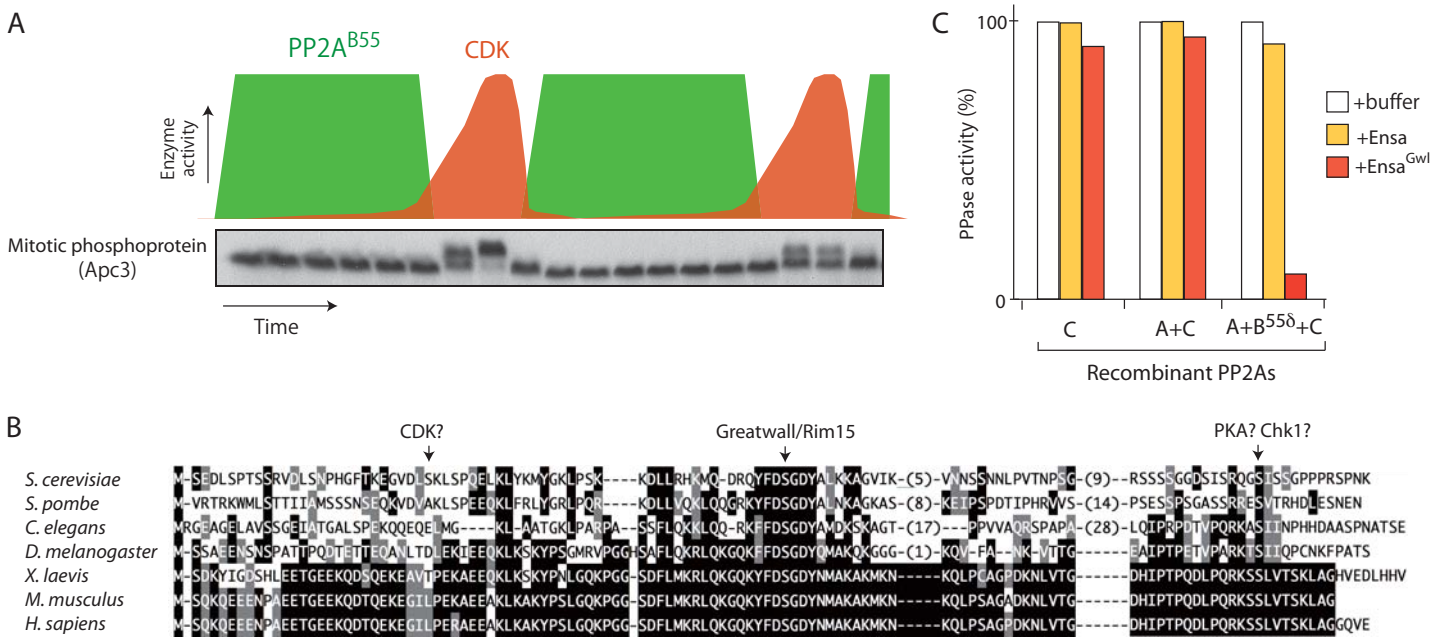


Figure 2, α-Endosulfine and ARPP-19 are Greatwall-dependent inhibitors of PP2A-B55

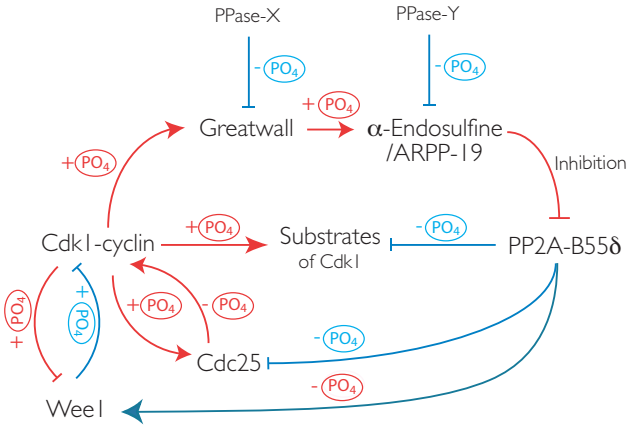


Figure 3: Factors that control the Gwl pathway during teh cell cycle