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Phosphatases and Kinases Regulating CDC25 Activity in the Cell Cycle: Clinical Implications of CDC25 Overexpression and Potential Treatment Strategies

Swastika Sur[1] and **Devendra K. Agrawal**[1,2,*]

[1]Department of Biomedical Sciences, Creighton University School of Medicine, Omaha, NE, USA

[2]Department of Clinical & Translational Science, Creighton University School of Medicine, Omaha, NE, USA

Abstract

Alterations in the cell cycle regulatory genes result in uncontrolled cell proliferation leading to several disease conditions. Cyclin-dependent kinases (CDK) and their regulatory subunit, cyclins, are essential proteins in cell-cycle progression. The activity of CDK is regulated by a series of phosphorylation and dephosphorylation at different amino acid residues. Cell Division Cycle-25 (CDC25) plays an important role in transitions between cell cycle phases by dephosphorylating and activating CDKs. CDC25B and CDC25C play a major role in G2/M progression, whereas CDC25A assists in G1/S transition. Different isomers of CDC25 expressions are upregulated in various clinicopathological situations. Overexpression of CDC25A deregulates G1/S and G2/M events, including the G2 checkpoint. CDC25B has oncogenic properties. Binding to the 14-3-3 proteins regulates the activity and localization of CDC25B. CDC25C is predominantly a nuclear protein in mammalian cells. At the G2/M transition, mitotic activation of CDC25C protein occurs by its dissociation from 14-3-3 proteins along with its phosphorylation at multiple sites within its N-terminal domain. In this article, we critically reviewed the biology of the activation/deactivation of CDC25 by kinases/phosphatases to maintain the level of CDK–cyclin activities and thus the genomic stability, clinical implications due to dysregulation of CDC25 and potential role of CDC25 inhibitors in diseases.

Keywords

CDC25 phosphatase; Kinases; Cell cycle; Cancer; Intimal hyperplasia

[*]Address for correspondence: Devendra K. Agrawal, Ph.D. (Biochem), Ph.D. (Med. Sciences), MBA, FAAAAI, FAHA, FAPS, FIACS, *Senior Associate Dean for Clinical & Translational Research*, Professor and Chairman, Department of Clinical & Translational Science, *The Peekie Nash Carpenter Endowed Chair in Medicine, Senior Associate Dean, Clinical & Translational Research*, Professor of Internal Medicine and Medical Microbiology & Immunology, CRISS II Room 510, 2500 California Plaza, Omaha, NE, 68178, USA, Tel: (402) 280-2938; Fax: (402) 280-1421, dkagr@creighton.edu.

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Introduction

Various pathophysiological conditions, including human malignancy, result due to altered cell-cycle regulatory genes leading to deregulated proliferation and cell cycle progression [1, 2]. Cyclin-dependent kinases (CDK) and their regulatory subunit, cyclins, play a critical role in cell-cycle progression. Various CDK/Cyclin complexes are consecutively activated and inactivated allowing the cell to progress through the cell cycle phases [3]. The activity of CDK is regulated by a series of phosphorylation and dephosphorylation of different residues. Cell Division Cycle-25 (CDC25) plays an important role in transitions between cell cycle phases by dephosphorylating these residues to activate CDKs [3, 4].

CDC25, dual specificity phosphatase (DSP), is an important checkpoint component, and dysregulation of CDC25 can contribute to genomic instability. Genomic instability occurs when cell continues to divide and the DNA lesion is passed to daughter cells due to defective cell cycle checkpoint. DSP is a sub-class of protein tyrosine phosphatases, which are both tyrosine- and threonine-specific [5, 6]. CDC25 phosphatases are found in all eukaryotic organisms except plants [7]. There are three isoforms of CDC25 in human, CDC25A, CDC25B and CDC25C. CDC25A is found to act at the G1/S transition. CDC25B and CDC25C play an important role at the G2/M transition [8, 9]. Different isomers of CDC25 expressions are upregulated in various clinicopathological conditions, including cancers. This upregulation may be the result of gene amplification or genetic mutation. Moreover, in some clinicopathological situation, such as neuroblastoma, 80% of the cases have overexpression of mRNA transcripts of CDC25 whereas only 5% of the cases showed an overexpression at the protein level [10]. This suggests that the CDC25 phosphatases get deregulated at any stage, such as transcription, translation, or the post-translational level. CDC25A is a transcriptional target of the E2F–RB1 pathway [11]. The Myc transcription factor initiates CDC25A protein expression and both Myc and CDC25A are expressed at elevated levels in tumors [8, 12,]. Transcription factors, E2F1/2/3 and STAT3, activate the transcription of CDC25s. Post-translationally, CDC25 undergoes phosphorylation and ubiquitination. The former may activate or inactivate CDC25 [14].

Structure of CDC25 Family

All three isoforms of CDC25 have molecular masses ranging between 53–65 KDa. CDC25A, CDC25B and CDC25C consist of 524, 580 and 473 amino acids [14]. The structure of CDC25 proteins can be divided into two main regions: the N-terminal region and the C-terminal region. The N-terminal region is extremely divergent and has sites for its phosphorylation and ubiquitination that regulate the phosphatase activity. The C-terminal is extremely homologous and contains the catalytic site [15]. The highly conserved region among the CDC25 family with unknown function is the modifiable cysteine residue, Cys484, located in a pocket binding to a sulfate group [16]. Hotspots, the key element for substrate recognition, are located about 20–30Å from the active site [17]. All the isomers of CDC25 have conserved catalytic domains, but quite diverse regulatory regions. Moreover, regulatory regions undergo alternative splicing events, which generate two variants for CDC25A and five each for CDC25B and CDC25C [18–21]. The non-catalytic domain determines the intracellular localization and turnover of the phosphatases.

CDC25A

CDC25A is considered an oncogene. Together, CDC25A and oncogenic ras can develop mammary tumors. E2F family is the transcription factor for CDC25A [11].

CDC25A during interphase and protein labile-stability state

CDC25A is labile in interphase and stable in mitosis. During interphase CDC25A is active, but it can abruptly switch from a labile to a stable protein while entering into mitosis. This switch is accompanied by uncoupling of CDC25A from the ubiquitin–proteasome-dependent degradation. CDC25A protein undergoes gradual complete destruction upon physiological dephosphorylation on exit from mitosis. These data suggest that mitosis-specific phosphorylation helps to build up the total activity of CDC25A through its stabilization. The entire checkpoints (G1, S, G2 and M) achieve the optimum sub-threshold levels of CDC25A by a switch of its 'labile' into the 'ultra-labile' state or ‘stable’ state, reflecting phosphorylation-induced increased activation of CDC25A. This concept of CDC25A protein stability switch can contribute to pathogenesis of some diseases, such as cancer. Overexpression of CDC25A deregulates G1/S and G2/M events, including the G2 checkpoint. CDC25A is highly expressed at late G1 phase and accelerates the entry into S-phase [14].

CDKs in Phosphorylation-dependent activation of CDC25A

In mitotic cells, cyclinB-Cdk1 phosphorylates CDC25A at Ser17 and Ser115 [22]. This phosphorylation by cyclinB-Cdk1 uncouples CDC25A from ubiquitin and prevents degradation. During mitosis, CDC25A is not ubiquitinated. This shows that uncoupling of CDC25A from the ubiquitin–proteasome-dependent degradation is important for the actively dividing cells. Phosphorylation leading to activation or inactivation of CDC25A occurs within the N-terminal regulatory region. There are possibilities that CyclinB-Cdk1 might phosphorylate CDC25A at other residues. Studies found that CDC25A, together with CDC25B and CDC25C, generates a cellular phosphatase pool required for full activation of cyclinB-Cdk1. CDC25A binds to cyclinB-Cdk1 *in vivo* and increases the endogenous cyclin B-Cdk1 kinase activity, thereby, modulating G2/M progression (Figure 1; Table 1). In vitro studies showed that in G1 phase, CDC25A is a positive regulator of CDK4 and CDK6. Later in G1 phase, CDC25A dephosphorylates the two inhibitory phosphorylation residues on CDK2 and activates it, promoting G1/S transition. The cell cycle–promoting action of CDC25A is tightly controlled in normal cells and mis-regulation of which may result in aberrantly high CDK activity [11, 13, 23]. Apart from the S-phase-promoting effect, CDC25A interacts with the main mitosis-promoting cyclin–CDK complex and generates a rate-limiting stimulus for the G2/M transition, and the lack of its activity can delay completion of the cell division cycle [22]. It was found that CDC25A phosphatase activates CDK2 and CDK1 by dephosphorylating Tyr15, which plays a key role in the RAS oncogenic pathway [11, 24]. Growth factors bind to HER2 and activate RAS-RAF-MAPK, which up-regulates cyclinD1 and downregulates p27Kip1 resulting in activation of CDK4, CDK6 and CDK2. CyclinD-CDK2 activates E2F transcription factor, which in turn increases the CDC25A and CHK1 level. The CHK1 mediates ubiquitinylated degradation of CDC25A

[11, 24]. In the following section, the key mediators and steps regulating CDC25A activity are described ((Figure 1; Table 1).

Phosphorylation-Dephosphorylation-dependent inactivation of CDC25A

CDK2-CyclinE—The phosphatase activity of CDC25A increases during S phase, which is essential for the GI-to-S phase transition. Studies confirmed that CDK2-cyclinE complex phosphorylates and activates CDC25A so that it can further dephosphorylate and activate CDKs required at the G1/S border [11, 13, 23].

CHK1—In a cell, the CDC25A level is strictly controlled by various efficient mechanisms. CHK1 targets CDC25A to ubiquitin-mediated degradation [11]. Chk1 phosphorylates CDC25A at Ser178, Ser296 and Thr507 mediating binding of 14-3-3 proteins to CDC25A impairing the ability of CDC25A to interact with cyclin B1/Cdk1 [25, 26].

CHK2—Upon DNA damage, at the S phase checkpoint activated CHK2 phosphorylates CDC25A at Ser123 resulting in ubiquitylation and degradation [27].

Casein kinase 1α (CK1α)—Casein kinase 1α (CK1α) phosphorylates CDC25A on both Ser79 and Ser82. CK1α can phosphorylate CDC25A, only upon prior phosphorylation of CDC25A at Ser76 by Chk1 or GSK-3β. This facilitates β-TrCP binding and ubiquitin-mediated proteolysis of CDC25A. The priming of CDC25A by at least three kinases, including Chk1, GSK-3β, CK1α, ensures diverse extra- and intracellular signals interfacing with CDC25A to precisely control cell division [28].

CK1ε—Unlike CK1α, CK1ε can directly phosphorylate Ser82 of CDC25A. Also, it is found that down-regulation of CK1ε stabilized the cellular CDC25A, indicating that CK1ε regulates the cellular levels of CDC25A [29].

ERK—In Xenopus eggs, when the extracellular signal-regulated kinase (ERK) pathway is strongly activated then it phosphorylate CDC25A for degradation. Both ERK and its downstream kinase p90rsk phosphorylate CDC25A, leading to ubiquitination by SCFβ-TrCP ubiquitin ligase causing proteosomal degradation. This suggests that strong ERK activation can target CDC25A for degradation in a manner very similar to Chk1 contributing to cell cycle arrest [30].

CDC14A phosphatases—Human CDC14A phosphatases dephosphorylate specifically the Cdk1/Cyclin-B1-dependent phosphate groups at Ser115 and Ser320 of CDC25A. Studies suggest that CDC14A may be involved in the cell cycle regulation of CDC25A stability [31].

Pathogenesis due to overexpression of CDC25A

CDC25A is considered an oncogene, as its over expression causes tumors in a variety of tissues. Higher expression of CDC25A was found in ovarian cancer patients, indicating that this CDK-regulating phosphatase can be a potential molecular target of novel drugs for ovarian cancer [32]. About 47% of human breast carcinoma cases are associated with overexpression of CDC25A. Inactivation of CDC25A by antisense oligonucleotides

inhibited the enzymatic activity of Cdk2 enzyme, arresting the G1 phase of cell cycle in human breast carcinoma cells [33]. This suggests that CDC25A-mediated activation of Cdk2 is required for S-phase entry in human breast cancer. In breast cancer, Myc protein expression is found to be upregulated, which further upregulates the expression of CDC25A [8,12]. In human esophageal cancer, overexpression of both protein and mRNA of CDC25A was found. Here, CDC25A activates cyclinA/E-Cdk2 by dephosphorylating them, resulting in the release of E2F transcription factor, which transcribes more of CDC25A [34]. CDC25A was also overexpressed in human hepatocellular carcinomas (HCC). The mechanism by which it is overexpressed in HCC is not clearly understood. Apart from its role in cell cycle regulation, CDC25A also inhibits cellular apoptosis [35].

Though there is high expression of CDC25A in 52 of 111 cases of human colorectal carcinoma, but no significant correlation with the disease was found [36]. Overexpression of CDC25A was found in vulvar carcinomas and also in normal vulvar squamous epithelium, suggesting that the expression of CDC25A is independent of the disease [37]. CDC25A phosphatase is a prominent stimulator of cell cycle progression and it plays a very basic role in the development of thyroid neoplasms [38].

CDC25B

CDC25B has oncogenic properties. CDC25B dephosphorylates at Thr14 and Tyr15 in the cyclin-dependent kinase CDC2 to make it active. This dephosphorylation is necessary for CDC2 to enter into mitosis. CDC25B has a nuclear localization signal (NLS) between amino acid residues 335 and 353 and a nuclear export sequence (NES) in the N-terminus (residues 28–40). CDC25B shuttles between the nucleus and the cytoplasm in an exportin-1-dependent manner. During the M/G1 phase of the cell cycle, CDC25B is nuclear and moves to the cytoplasm during S and G2 [39]. The cytoplasmic localization of CDC25B depends on NES, whose mutation causes CDC25B to partially lose its activity as a mitotic inducer [39] (Figure 2; Table 2).

CDC25B in the interphase

During the interphase in the cell cycle, the CDC25B is located in the nucleus. At the end of G2 phase of cell cycle, CDC25B from the nucleus translocates to the cytoplasm, where it activates CDK1/cyclinB. It is found that CDC25B is phosphorylated and activated in the late S-phase in the nucleus, but is translocated to the cytoplasm only in the late G2 phase. The unstable CDC25B has short half-life and undergoes proteosomal degradation. Mitosis is blocked in the absence of CDC25B since both CDC25C and CDC25B are required for full activation of CDK1/cyclinB in the G2 phase. This activation of CDK1/cyclinB in the G2 phase leads to the initiation of early prophase of the cytoskeleton. During prophase the activated CDK1/cyclinB are transferred to the nucleus, where it starts the positive feedback loop by further activating CDC25B [40]. Binding to the 14-3-3 proteins regulates the activity and localization of CDC25B. CDC25B has three binding sites, Ser151, Ser230, and Ser323, but 14-3-3 proteins binds to the Ser323 residue of CDC25B with the highest affinity. This decreases cyclin/Cdk substrate access to the catalytic site of CDC25B, thus down-regulating its activity. Phosphorylated Ser323 is maintained into mitosis, but phosphorylation of Ser321

disrupts the binding of 14-3-3 proteins to Ser323 (Figure 2; Table 2). Phosphorylation of Ser321 by Cdk1 disrupts the binding of Ser323 to the 14-3-3 proteins, thus fully activating CDC25B [41].

Phosphorylation-dependent activation of CDC25B

Aurora-A kinase: It phosphorylates CDC25B at Ser353 and causes its entry into mitosis. The phosphorylated CDC25B is found to be located at the centrosome from prophase to anaphase. Aurora-A co-localizes with phosphorylated CDC25B at the centrosome and the poles of the mitotic spindle. Aurora-A is not activated and consequently CDC25B is not phosphorylated when G2/M checkpoint is activated by DNA damage [42, 43].

Polo-like kinase 1 (PLK1): The CDC25B phosphatase with polo-like kinase 1 (PLK1) activates CDC2 and regulates the G2 phase of the cell cycle progression. PLK1 activity is essential for the relocation of CDC25B from the cytoplasm to the nucleus at the G2-M transition regulating its mitosis-inducing activity [44]. The amino acid residues at Ser50, Thr58, Thr127, Thr167, Ser209, Thr265, Ser291, Ser353, Ser375, Ser397, Thr404, Ser465 and Ser513 are the thirteen different sites on CDC25B phosphorylated by PLK1 [45].

Protein kinase A (PKA): In fertilized egg of mice, Ser321 and Ser229 of CDC25B are the two potential phosphorylation sites for the activity of the catalytic subunit-α of protein kinase A, which is encoded by the *PRKACA* gene (PRKACA), with Ser321 being the primary site. PRKACA phosphorylates and activates CDC25B in fertilized eggs of mice [46]. Inhibition of PRKACA in the interphase in Xenopus egg extracts caused rapid onset of mitosis, whereas stimulation of PRKACA arrested interphase by down-regulating CDC25 activity [47]. Therefore, PRKACA may act as a negative/positive regulator of CDC25B. It is also possible that PRKACA phosphorylates the Ser321 of CDC25B, which then binds to 14-3-3 proteins in the cytoplasm at the G1 and S phase. In the G2 phase, CDC25B initiates the mitosis after the removal of 14-3-3 proteins. Overall, PRKACA may directly phosphorylate CDC25B on Ser321 to control cell cycle progression in mouse-fertilized eggs [46]. In G2/M transition of fertilized mouse eggs, Ser149 may be another potential PKA phosphorylation target of CDC25B [48]. In mammalian oocytes, CDC25B is a direct target of PKA, which phosphorylates CDC25B at Ser321 resulting in its inhibition and sequestration by the 14-3-3 proteins [49].

Phosphorylation-Dephosphorylation-dependent inactivation of CDC25B

MAPK5—The MAPK5 pathway comprises of Ras, Raf, MEK, and ERK that provides the mechanism to convert extracellular signals into intracellular response. ERK-dependent phosphorylation is the primary signaling output of the pathway. These pathways target the CDC25 phosphatase family (Figure 2; Table 2). MEK1 destabilizes the CDC25B by phosphorylating the Ser249, leading to proteosomal degradation of CDC25B. Down-regulation of CDC25B causes significant delay in entry into mitosis [50].

JNK and p38—Under cellular stress, activated JNK and p38 phosphorylate Ser101 of CDC25B, resulting in the rapid degradation of CDC25B and cell cycle arrest. The SCFβ-TrCP–mediated ubiquitinylation -proteasome pathway degrades CDC25B [51].

Checkpoint kinase (Chk1)—During interphase, the checkpoint kinase Chk1 is located in the centrosome, from where it dissociates at the onset of mitosis. Chk1 causes centrosome-associated inhibitory Tyr15 phosphorylation of Cdk1, which gets activated in the late prophase by the cytoplasmic CDC25B. CDC25B, in turn, is negatively regulated by centrosome-associated Chk1. Chk1 phosphorylates CDC25B at S230 and S563. Checkpoint defects and mitotic abnormalities are often found in cancer; therefore, dysfunctional Chk1 might contribute to these cancer-associated defects [52, 53].

CDC14A phosphatase—CDC14A phosphatase dephosphorylates CDC25B at Cdk1-cyclin B1 phosphorylation sites located within its N-terminal domain, inhibiting its catalytic activity. Thus, CDC25B is a new substrate of CDC14A. This inhibitory effect of CDC14A by dephosphorylation of CDC25B leads to a decrease in Cdk1 activity at the onset of mitosis. More studies are warranted to understand whether the regulation of CDC25B activity by CDC14A occurs in centrosomes or the centrosome-released pool of CDC14A at early mitosis performs it. This shows that in human cells CDC14A plays a role in preventing premature entry into mitosis [54].

Pathogenesis due to overexpression of CDC25B

CDC25B is an oncogenic protein and induces neoplastic transformation. In human colorectal carcinoma, CDC25B is overexpressed to activate the CDC2/cyclinB complex and to enhance the growth and survival of these tumors [36]. In a study with 106 ovarian cancer patients, CDC25B was expressed in all the samples and was found to be associated with poor prognosis of the cancer [32]. Only 16% of the vulvular carcinoma patients were found to have higher expression of CDC25B than normal vulvar squamous epithelium. This suggests that CDC25B is associated with tumorigenesis in a small number of vulvular carcinoma [37]. In mammary glands of transgenic mouse, overexpression of CDC25B is found to generate mammary gland hyperplasia. The higher expression of CDC25B is found to be associated with increased expression of cyclinD1 protein and cyclinE/cdk2 activity to increase proliferation, and also with a decreased expression of c-myc and p53 to reduce apoptosis [55]. In case of human gastric carcinoma, 70% of the tumors overexpressed CDC25B mRNA causing development, progression and invasion of tumor cells leading to malignancy [56]. In human prostate cancer, CDC25B is overexpressed and interacts with co-activators of androgen receptor (AR) and enhances AR-mediated transcription, causing the development of prostate cancer [57]. CDC25B enhances the early phase of progression of human thyroid carcinoma [38]. In case of non-small cell lung cancer (NSCLC) and ovarian cancer patients, higher CDC25B expression had significantly poor survival compared to the patients with low CDC25B expression [32, 58].

CDC25C

CDC25C gene is highly conserved during evolution. CDC25C is predominantly a nuclear protein in mammalian cells. It plays a key role in the regulation of cell division. It dephosphorylates the Thr14 and Tyr15 residue of cyclinB-bound CDC2 and triggers entry into mitosis. It is also believed that it suppresses p53-induced growth arrest. CDC25C is

found to have many spliced transcript variants, however, not much is known about their function.

CDC25C-Interphase

During interphase, CDC25C has low phosphatase activity. Phosphorylation of CDC25C on Ser216 in interphase prevents its activation, and promotes its sequestration in the cytoplasm through association with 14-3-3 proteins [59–61]. In the G2/M transition, mitotic activation of CDC25C protein occurs by its dissociation from 14-3-3 proteins, together with its phosphorylation at multiple sites, Thr48, Thr67, Ser 122, Thr130 and Ser214, within its N-terminal domain [62–66]. During mitosis in human, phosphorylation of Ser214 on CDC25C prevents its phosphorylation of Ser216 and inactivation by binding to 14-3-3 proteins [62]. In Xenopus, phosphorylation of Thr130 on CDC25C is needed to dissociate from 14-3-3 proteins [67–69]. Xenopus CDC25C undergoes a major shift in electrophoretic mobility due to extensive phosphorylation with increased phosphatase activity [70, 71]. CDC25Cs are found to either exist in hypo-phosphorylated inactive form or hyper-phosphorylated active form, but growing evidence suggests that intermediate phosphorylation variants might also be present [62, 67–69]. Both full-length and splice variants of CDC25C with different phosphorylation pattern are present in mitotic phase revealing that CDC25C has distinct biological functions [72]. Splice variants of CDC25C are phosphorylated on Ser214 and on Thr48 in mitosis, implying that they might have similar regulatory mechanism as full-length CDC25C [73]. Recently, it is found that during human mitosis, different phosphorylated forms of CDC25C are associated with different partner proteins and have differential spatial organization. The non-phosphorylated mutant forms of CDC25C impair mitotic progression in human cells. This finding contradicts auto-amplification mechanism involving CDC25C and Cdk1 in mitotic activation [74] (Figure 3; Table 3).

Phosphorylation-dependent activation of CDC25C

Several kinases phosphorylate human CDC25C during mitosis. In human, CDC25C also found to play a similar role in meiotic cells. During meiosis, CDC25C is phosphorylated and localized just like during mitotic divisions. This suggests that the involvement of CDC25C is conserved and functional in meiotic cells [75] (Figure 3; Table 3).

Polo-Like Kinase—The polo like kinases (PLK) belongs to the family of serine/threonine kinases and is found to play major role in controlling mitotic events in human [64]. Xenopus homologue, PLX1, is identified as the first kinase other than CDC2 that phosphorylates XCDC25C at multiple sites to increase its activity [76]. Human PLK phosphorylates and activates CDC25C, which in turn dephosphorylates inactive CDC2/CyclinB into active form to progress the cell cycle [64]. During prophase in vertebrate cells, M-phase promoting factor (MPF), such as CDC2-cyclinB1, should be translocated to the nucleus from the cytoplasm for coordinating M-phase events. PLK1 is found to phosphorylate S147 and/or S133 of CDC2-CyclinB1 to stimulate its entry to the nucleus [77]. Also, PLK1 phosphorylates CDC25C at Ser198 and stimulates nuclear translocation of CDC25C during prophase [78]. PLK3 also phosphorylates Ser191 causing translocation of CDC25C [79].

Cyclin-dependent Kinases (CDKs)—CyclinB-complexed-p34CDC2 protein kinase phosphorylates CDC25C. CyclinB-complexed-p34CDC2 protein kinase and CDC25C are part of a positive autocatalytic activation loop, in which CDC25C activates CyclinB-complexed-p34CDC2 protein kinase to phosphorylate CDC25C and activate further p34CDC2-cyclinB kinase. First, the CDC25C removes the inhibitory phosphatases from Thr14 and Tyr15 of CDC2 to activate it. The activated CDC2/CyclinB phosphorylates at Thr48, Thr67, Thr138, Ser205 and Ser285 of CDC25C and activates it. This entire event of phosphorylation and activation of both CDC2/CyclinB-CDC25C either occurs in nucleus or takes place only after CDC25 has translocated to the cytoplasm [63].

Calcium/calmodulin-dependent protein kinase (CaM kinase II)—Calmodulin inhibitor attenuates the cell cycle progression at G2 phase. Also, during M phase, CDC25C is phosphorylated in mammalian cells, which can be inhibited by KN-93, an inhibitor of CaM kinase II. KN-93-induced inhibition of phosphorylation of CDC25C inhibited progression of the cells through the G2/M phase checkpoint. CDC25C protein is a substrate for CaM kinase II, and its phosphorylation increases its phosphatase activity. Amino acid residues Ser38, Ser216, Ser449, and Ser451 are the four CaM kinase II substrate consensus sequences on human CDC25C protein. Multifunctional Ca^{2+}/CaM-dependent protein kinase phosphorylates CDC25C on these serine residues and enhances its phosphatase activity [66].

PP1—PP1 directly interacts with CDC25C since it has a docking site in the N-terminus of CDC25. After removal of the CDC25C inhibitory 14-3-3 proteins, PP1 dephosphorylates CDC25C at Ser216 upon entry in the M-phase and activates it [67].

Phosphorylation-Dephosphorylation-dependent inactivation of CDC25C

Nature does not leave anything uncontrolled in the living world. Therefore, various proteins also inactivate CDC25C (Figure 3; Table 3). These are discussed in the following section.

Checkpoint Kinase (Chk1)—Human Chk1 is involved in DNA damage checkpoint responses. It phosphorylates CDC25C at Ser287 and negatively regulates CDC25C, thus controlling the cell cycle checkpoint. Cds1/Chk2 and the c-TAK1 kinase can also phosphorylate CDC25C at Ser287 to inactivate it. Followed by the binding of 14-3-3 proteins to CDC25C, CDC2 or CDK1 phosphorylates Thr138 on CDC25C, causing dissociation of 14-3-3 proteins from CDC25C. At this point PP1 may come at the site to induce dephosphorylation at the Ser287 on CDC25C, thus activating it [67].

PP2A and its subunit B56δ—Phosphatase PP2A with its subunit B56δ negatively regulates CDC25C activity by dephosphorylating Thr138, leading to its exit from mitosis. Chk1 phosphorylation of B56δ enhances PP2A-mediated Thr138 dephosphorylation. B56δ binds to and inactivates CDC25C by dephosphorylating the site (Thr138) needed for mitotic function. The inactive CDC25C then gets phosphorylated at Ser216 by Chk1, followed by the attachment of 14-3-3, and gets sequestered in the cytoplasm. It is possible that there are many other events that regulate the activity of PP2A-B56δ-induced dephosphorylation of CDC25C [59, 69].

Human CDC14B phosphatase (hCDC14B)—The role of hCDC14B in cell cycle regulation is controversial. It is released in prometaphase and involved with nucleolar chromatin during interphase. Overexpression of hCDC14B delays the activation of Cdk1/cyclinB and dephosphorylates or inactivates mitotic CDC25 proteins. Depletion of hCDC14B by siRNA leads to hyperphosphorylation of CDC25 and increased Cdk1 activity [80].

c-Jun NH(2)-terminal Kinases (JNKs)—During G2 phase of the cell cycle JNK phosphorylates CDC25C at Ser168 and negatively regulates its phosphatase activity and thereby Cdk1 activation, thus controlling the onset of mitosis [81].

Pathogenesis due to overexpression of CDC25C

CDC25C appears to play a lesser oncogenic role than CDC25A and CDC25B, as only limited cancers have an elevated level of CDC25C expression. In human prostate cancer, high expression of both CDC25C and an alternatively spliced variant was observed at the transcription level [79]. Reduced level of inactive phosphorylated form of CDC25C was bserved with a marked increase in CDC25C phosphatase activity in prostate cancer. More studies are needed to determine the role of CDC25C and its spliced variants in the pathogenesis of prostate cancer [82]. In the development and progression of human vulvar carcinomas, CDC25C and phospho-CDC25C (Ser216) were found to play a significant role [37].

CDC25 As A Target For Cancer Treatment

As discussed above, all isoforms of CDC25 are tightly regulated throughout different phases in the cell cycle. Overexpression of CDC25 leads to over-activation of several CDKs resulting in cell cycle progression in an untimely manner by disregarding the checkpoint barrier. This contributes to cancer development and genomic instability. CDC25 phosphatases act as a hub, where it receives several mitogenic signals and facilitate cell cycle progression. This crucial role of CDC25 in the control of cell cycle makes it an ideal target for cancer treatment. This is further supported by a study in yeast showing cell cycle arrest due to CDC25 inhibition.. However, one concern could be that any CDC25 inhibitor will stop the cell cycle progression of any cell in an unselective manner. In a study in colon adenocarcinoma cell lines, it was found that cells with overexpressed CDC25 were more sensitive towards its inhibitors than normally dividing cells. A combinational therapy with paclitaxel has shown a promising therapeutic potential in cancer cells. Accordingly, selective inhibition of CDC25 could be a therapeutic option, In the following section, so far identified CDC25 inhibitory compounds are discussed [8].

Natural and Synthetic Inhibitors of CDC25

As discussed above, a balance between cyclins, cyclin-dependent kinases (Cdks) and phosphatases control, at least in part, the cell cycle progression and cell proliferation. Loss in basic cell cycle regulation may be caused by cancer-associated mutations, loss of CDK inhibitor expression and/or overexpression of cell cycle-regulated protein. Few potent natural and synthetic inhibitors of CDC25s have been discovered so far. Depending on their

behavior, these can be classified into reversible and irreversible inhibitors. Most of the reversible inhibitors belong to chemical classes, including bioisosteres and electrophilic entities and the family of indolyl-1,4-hydroxyquinones. Menadione vitamin K3, NSC663284, caulibugulones, and BN82002 are few of the irreversible inhibitor of CDC25s. Irreversible inhibitors inhibit the active site of CDC25s through a covalent modification of the active site or an oxidation of the catalytic cysteine [14, 83].

Some of the CDC25 inhibitors, known so far, include compound 5169131, KR61639, analogues of cryptotanshinones and miltirone, compound LGH00031, NSC 95397, and others. The compound 5169131 arrests G1/S and G2/M with IC_{50} values of 5, 10.4, and 8.8 μmol/L against CDC25A, CDC25B, and CDC25C, respectively [81]. KR61639 inhibits CDC25B (IC_{50}: 0.67μmol/L). Cryptotanshinones and miltirone analogues are moderate inhibitors of CDC25B phosphatase (IC_{50}: 3.2–24 μmol/L). However, the mechanisms of action of these inhibitors are unclear. Compound LGH00031 is an irreversible inhibitor of CDC25B with IC_{50} values of 0.143–0.328 μmol/L and inhibits the proliferation of A549, HeLa, and HCT116 cells [81]. LGH00031 may interact with CDC25B to increase ROS concentration around CDC25B and make it highly specific for CDC25. *In vitro* studies with this inhibitor of CDC25 revealed strong effects against cancer cells suggesting that it is a potent CDC25 inhibitor. Further *in vivo* studies are required to establish LGH00031 as an efficient CDC25 inhibitor [84]. 2-fluoro-4-hydroxybenzonitrile, a CDC25B inhibitor, binds to its catalytic domain and prevents protein-protein interaction with CDK2/Cyclin A [85]. Anti-cancer drug, NSC 95397, inhibits CDC25A and decreases NF-kB-mediated NO production [86].

Irreversible inhibitor, NSC 119915, generates intracellular ROS in cells, arrests cells in the G0/G1 and G2/M phases of the cell cycle by inhibiting CDC25A and CDC25B. This inhibitor significantly suppresses the growth of K562 leukemia, PC-3 prostate and MCF-7 breast cancer cell lines [14].

Imidazopyridine derivative, CHEQ-2, induces S-phase cell cycle arrest by suppressing CDC25A/B expression leading to activation of apoptosis. Cell apoptosis in CHEQ-2-treated cells is caused by increased ROS level and apparent decline in membrane potential. The 10 mg/kg of oral administration of CHEQ-2 in nude mice significantly inhibited xenografted human liver tumor growth. CHEQ-2 is a novel CDC25 inhibitor with remarkable tumor inhibition activities by inducing cell cycle arrest, apoptosis, ROS production and mitochondrial dysfunction [87].

Novellino and colleagues [85] identified molecules that inactivate/inhibit CDC25B at micromolar concentration in vitro, inhibit breast (MCF-7), prostate (PC-3), and leukemia (K562) cancer cell proliferation, and significantly affect the cell cycle progression. These molecules were identified by an experimental and virtual screenings of both National Cancer Institute (NCI) Diversity Set and ZINC34 databases. Based on the in vitro assay for CDC25A, CDC25B and CDC25C activity, fifteen CDC25 inhibitors were selected. However, compound 11 (irreversible inhibitor), compound 12 (reversible inhibitor), compound 18 and compound 19 (inhibitors with intermediate behavior) were selected for detailed studies. These structurally distinct inhibitors were obtained by the combination of

computational, biochemical, and cell biology experiments, and all these compounds significantly inhibited MCF-7, PC-3, and K562 cell proliferation [88].

SV37 is another CDC25 inhibitor that efficiently inhibits all three purified human CDC25 isoforms (IC_{50} 1–9mM). Its structure is based on both coumarin and quinone moieties. In breast cancer cells, it generates ROS and accumulates pCDK leading to apoptosis in the triple negative MDA-MB-231 cells [89]. Given the critical role played by CDC25 in cell survival pathways, a further fine-tuning of these compounds may dictate better therapeutic potential.

The bis-quinonoid IRC-083864, under the name of Debio 0931 and is currently in the clinical trial, is a potent CDC25 inhibitor with an IC_{50} value of 20 nM [14]. Another compound, BN 82685, alone may inhibit all three CDC25 phosphatases in human pancreatic tumor Mia PaCa-2 cells. In combination with paclitaxel, it inhibits the proliferation of colon cancer cells [14]. DADA 3003-2 is a compound obtained from the compounds library at the University of Pittsburgh, and is found to hyperphosphorylate G2/M cyclin-Cdk complex resulting in G2/M accumulation.

In early and advanced stage prostate cancer, CDC25B directly acts as the co-activator and CDC25A acts as the co-repressor of androgen receptor. It is challenging to use previously screened CDC25 inhibitors as molecular targeting agents for androgen co-regulators in androgen refractory prostate cancer. As CDC25 is one of the critical targets for androgen refractory prostate cancer treatment, the clinical application of the CDC25 inhibitor as an anti-cancer drug is expected in the future [90]. A study used xenograft molecular apocrine model to demonstrate that with combination therapy breast cancer patients may get significantly better therapeutic response compared to monotherapy. They showed that the combined application of androgen receptor and CDC25A inhibitors are promising therapeutic strategy in molecular apocrine breast cancer [91].

Conclusion and Future Direction

The above information highlights the importance of the tight regulation of CDC25 phosphatases throughout the cell division cycle to maintain normal cell cycle progression and proliferation. During normal cell cycle, CDC25 phosphatases must respond to the activated checkpoint and stop the cell cycle progression to initiate apoptosis or DNA repair. Any kind of aberrant regulation of these control mechanism contributes to tumorigenesis or uncontrolled proliferation of the cells, such as intimal hyperplasia leading to restenosis in cardiovascular diseases. The overexpression of CDC25 is associated with different clinicopathological conditions. Isoform-specific overexpression of CDC25 in different clinicopathological conditions occurs through independent pathways. Inhibition of this phosphatase may be a promising anticancer strategy. Majority of the most potent inhibitors of CDC25s have toxic side effects, thus limiting their therapeutic applications. Few of the inhibitors attenuate the phosphatase activity of all three isoforms of CDC25s in an unselective manner. Future studies are required to develop novel molecules that have more potent inhibitory activity on CDC25 without any adverse effects, and thus regulating the uncontrolled proliferation of various pathological conditions.

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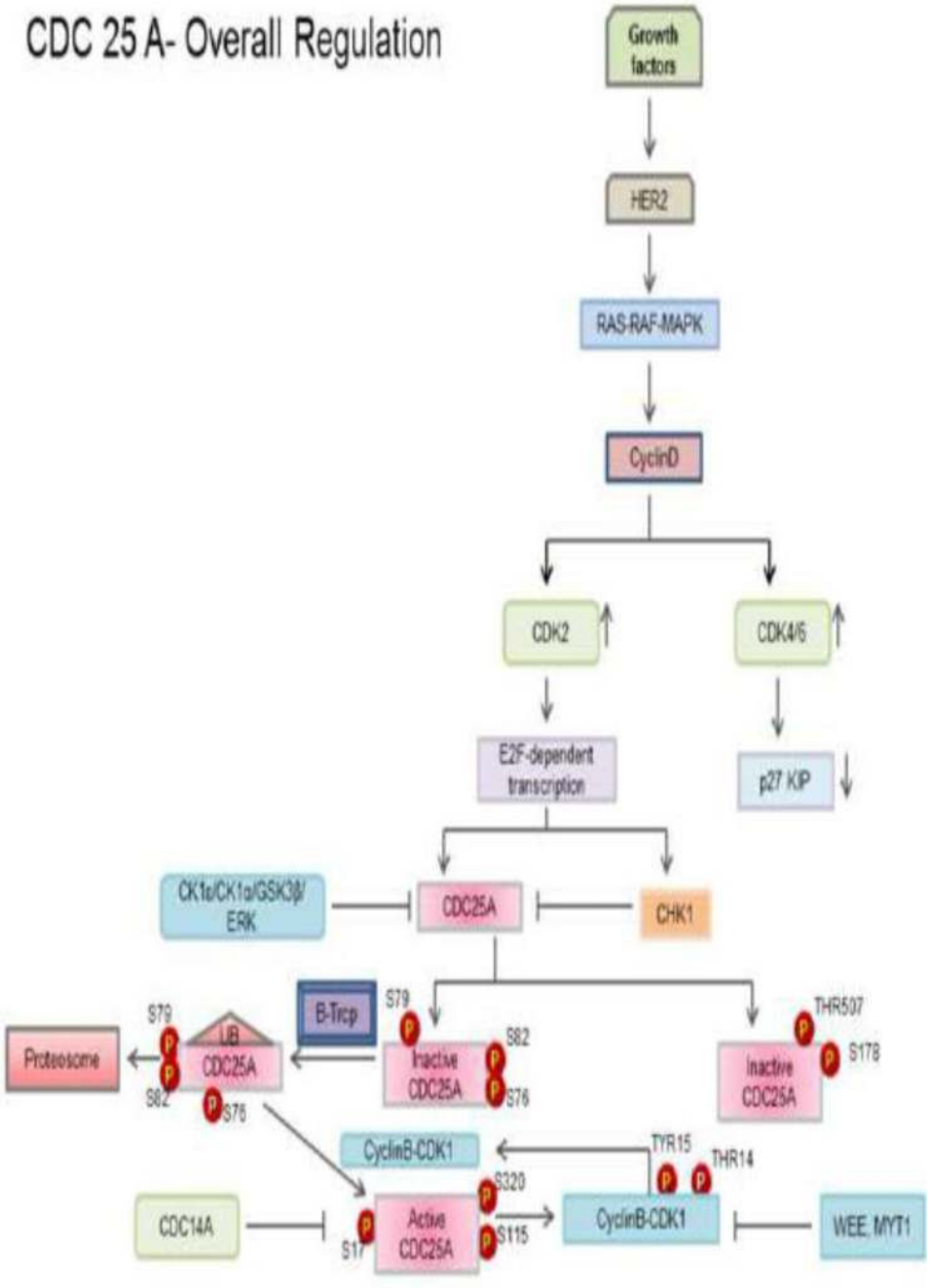

Fig. 1.

A simplified schematic representation of molecular interactions that regulate CDC25A activity and expression. Note that CDC25A and CyclinB-CDK1 mutually activate each other. The expression of CDC25A is upregulated by transcription factor, E2F. CHK1, CK1ε, CK1α, GSK3β, and ERK inhibit the activity of CDC25A. B-Trcp ubiquitinates CDC25A and leads to its proteasome-mediated degradation. The key phosphorylation sites leading to the activation and inactivation of CDC25A are depicted in red.

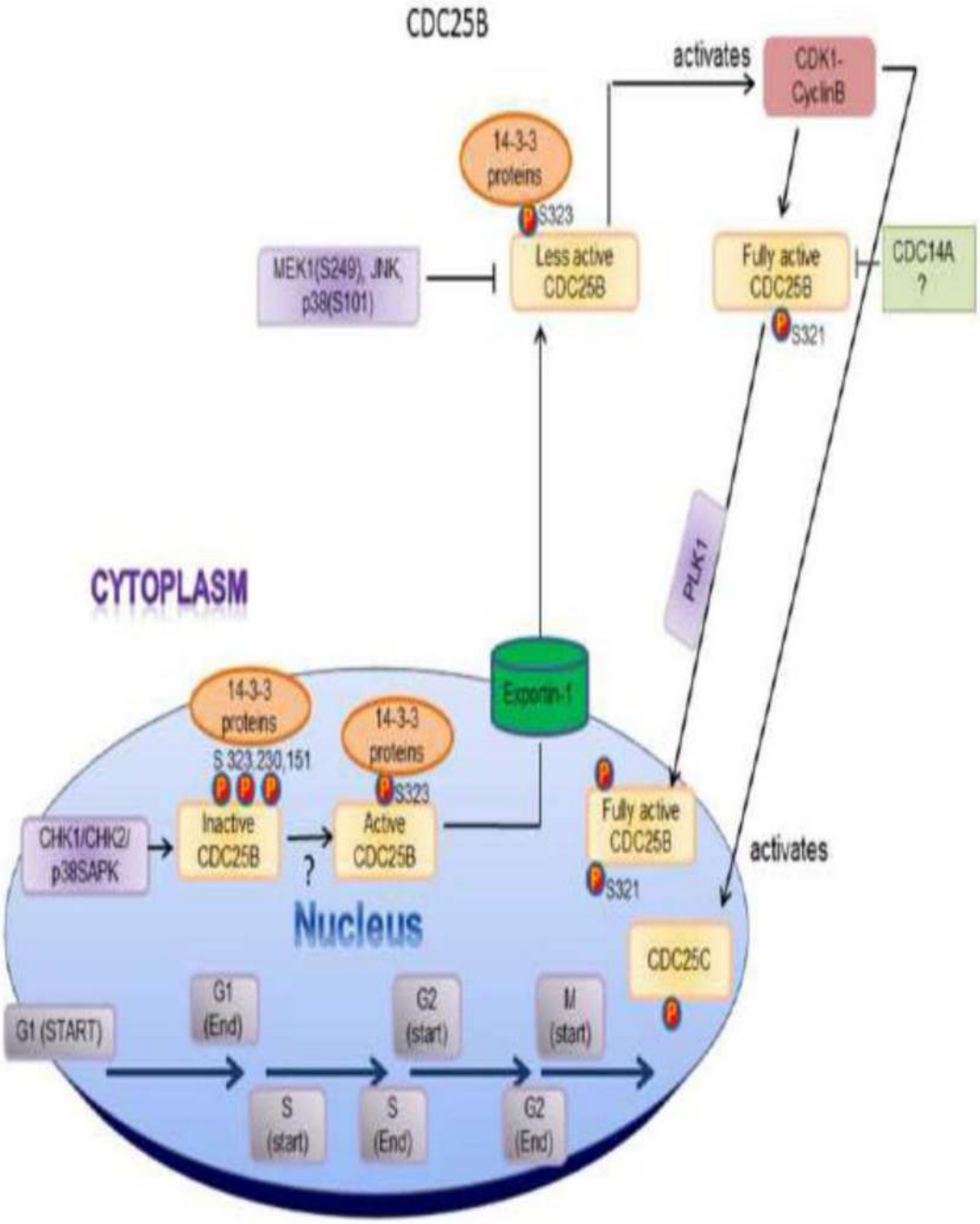

Fig. 2.

Molecular interactions regulating CDC25B activity, which in turn regulates the cell cycle. CHK1, CHK2, P38SAPK, MEK1, JNK and p38 phosphorylate and inactivate CDC25B, which when uncoupled from 14-3-3 proteins undergoes full activation in the cytoplasm. CDK1-CyclinB and PLK1 phosphorylate and activate CDC25B causing its translocation to the nucleus

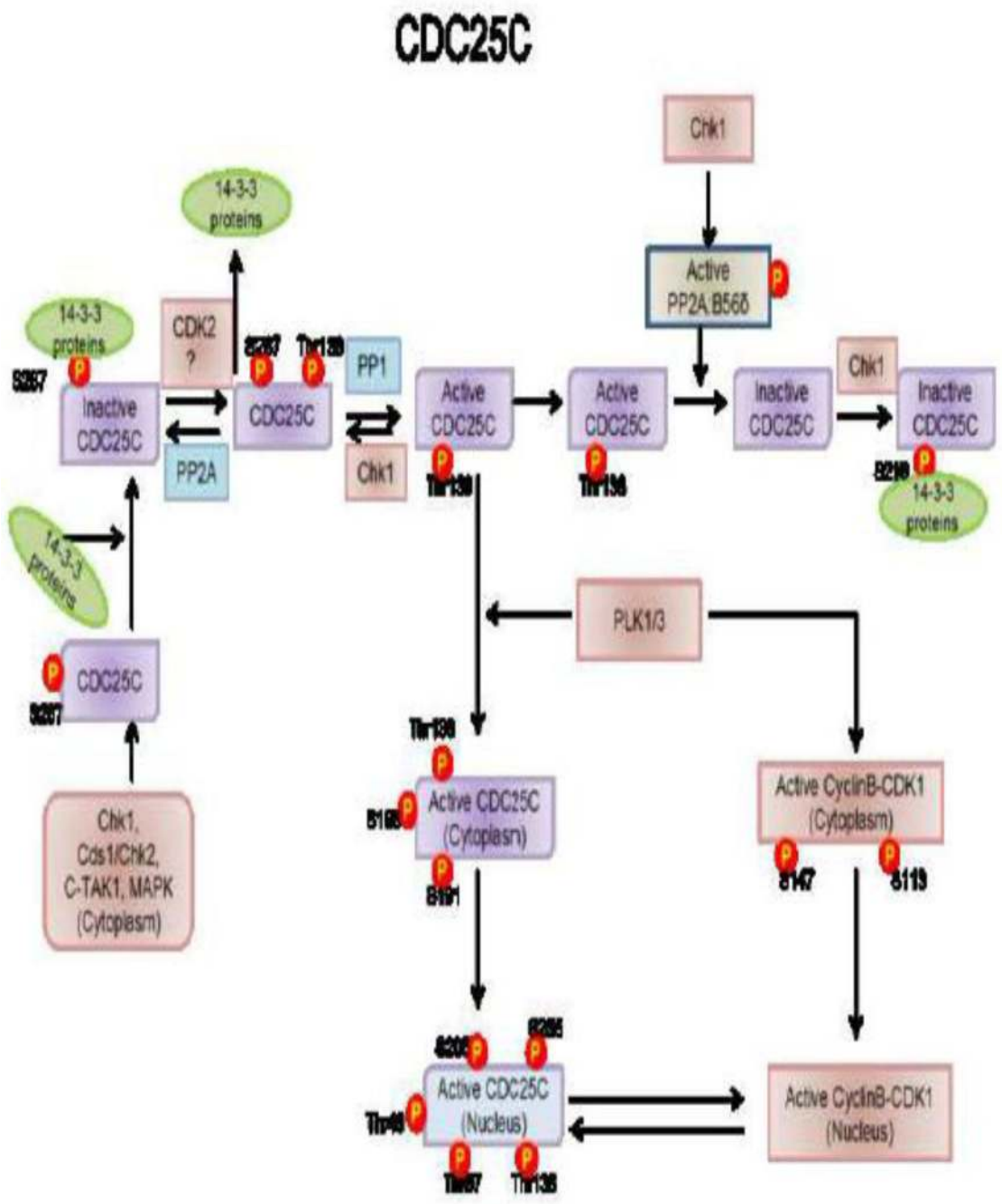

Fig. 3.

An overview of the molecular interactions that regulate the activity of CDC25C. Chk1, Cds1/Chk2, C-TAK1, MAPK, CDK2, PP2A inactivate CDC25C. Phosphatase, PP1, dephosphorylates and activates CDC25C. PLK1 and CyclinB-CDK1 further activate CDC25C by phosphorylating it. Note that the key phosphorylation sites leading to the activation and inactivation of CDC25C are depicted in red.

Table 1

Kinases and phosphatases regulating the activity of CDC25A.

Target protein	Responsible kinase/phosphatases	Phosphorylation site	Potential function
CDC25A	CyclinE-Cdk2		Phosphorylation-mediated activation
	CHK1	Ser178, Ser296, Thr507, Ser76	14-3-3 protein mediated inactivation of CDC25A
	CHK2	Ser123	CDC25A degradation
	CK1α	Ser79, Ser82	Proteolysis of CDC25A
	GSK-3β	Ser76	Priming of CDC25A to precisely control cell division
	CK1ε	Ser82	Ubiquitin-mediated proteolysis
	ERK	Ser85	Degradation of CDC25A in Xenopus
	CDC14A phosphatase	Ser115, Ser320	Dephosphorylates CDC25A

Table 2

Kinases and phosphatases regulating the activity of CDC25B

Target protein	Responsible kinase/phosphatases	Phosphorylation site	Potential function
CDC25B	**Aurora-A kinase**	Ser353	Entry to mitosis
	PLK1	Ser50, Thr58, Thr127, Thr167, Ser209, Thr265, Ser291, Ser353, Ser375, Ser397, Thr404, Ser465 and Ser513	Mitotic inducing activity
	Protein kinase A	Ser321, Ser229	Activates CDC25B in mouse fertilized eggs
	MAPK5	Ser249	Proteosomal degradation of cdc25B
	JNK and p38	Ser101	Proteasomal degradation of CDC25B
	Chk1	S230 and S563	Negatively regulates CDC25B activity
	CDC14A phosphatase		Inactivates CDC25B

Table 3

Kinases and phosphatases regulating the activity of CDC25C

Target protein	Responsible kinase/phosphatases	Phosphorylation site	Potential function
CDC25C	PLK 1	Ser198	Nuclear translocation of CDC25C
	CDC2/cyclin B	Thr48, Thr67, Thr138, Ser205 and Ser285	Activates CDC25C
	CaM kinase II	Ser38, Ser216, Ser449, and Ser451	Activates CDC25C
	PP1	Ser216	Dephosphorylate CDC25C
	Chk1	Ser287	Negatively regulates Cdc25C
	PP2A:B56δ	Thr138	Inactivates Cdc25C
	Human CDC14B phosphatase		Inactivates CDC25C
	JNK	Ser168	Inactivates CDC25C