

Cdc25 protein phosphatase: regulation and its role in cancer

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Summary

The family of the Cdc25 dual-specific protein tyrosine/threonine phosphatases is critically involved in cell cycle control. The substrates of Cdc25 are cyclin-dependent kinases, which are regulated by the phosphorylation of threonine and tyrosine residues. Cdc25 regulation and activity reveals a complex network of counter-balancing mechanisms and puts it on the crossroads of fundamental cellular events like cell proliferation, cell cycle arrest and apoptosis. Our present knowledge of the biology and biochemistry of Cdc25 phosphatases makes them attractive targets for drug discovery efforts: (a) they phase critical, non-redundant cell cycle regulatory functions; (b) they are *bona fide* checkpoint genes; (c) they have tight substrate specificities and a well-defined mechanism of catalysis; (d) they are potential targets of at least two oncogenes (Raf1 and c-Myc) that are frequently altered in human cancers; (e) they co-operate with other oncogenes in cell transformation and thus are *bona fide* proto-oncogenes; and lastly (f) their expression is altered in tumors.

I. Introduction: Cdc25 and the cell cycle

The role of Cdc25 as an inducer of mitosis first emerged from studies of yeast genetics that linked the phosphorylation state of the Cdc2 cyclin-dependent kinase to the activity of a protein phosphatase (Russell and Nurse, 1986). Later, the gene product of the *cdc25* gene was identified to be a dual-specificity phosphatase that removes inhibitory phosphorylations of Cdc2, both from a highly conserved tyrosine residue (Tyr15) and a less conserved threonine residue (Thr14, **Figure 1**). Homologs of the yeast gene were identified in a wide variety of organisms. This functional conservation of Cdc25 throughout evolution illustrates its fundamental role in controlling the cell cycle.

The regulation of proteins of the cyclin-dependent kinase (Cdk) family has been studied in great detail, and several *cdc25* genes have been identified in mammals. In humans the three homologs that were isolated are Cdc25A, B and C. Cdc25 C is the mitotic inducer; its substrate is the hyperphosphorylated complex of Cdc2/cyclin B. The functions of Cdc25A and B are less clear, with their possible substrates ranging from Cdk4/cyclin D (Terada et al., 1995), Cdk2/cyclin E and cyclin A complexes (Hoffmann et al., 1994) to

Cdc2/cyclin A and cyclin B complexes (for a recent review on Cdc25 cell biology and biochemistry, see Draetta and Eckstein, 1997).

Recent investigations into the regulation of Cdc25 itself are beginning to shed light on an intriguing and complex network of players (please refer to **Figure 2** throughout the text). They place Cdc25 squarely on the crossroads between cell proliferation, apoptosis, mitogenic signal transduction, and cancer. This chapter reviews briefly the emerging understanding of Cdc25 regulation and its implications for human cancer.

II. Cdc25 is a phosphoprotein

The Cdc25 protein undergoes phosphorylation during the cell cycle (Izumi et al., 1992), a step that triggers its phosphatase activity. The phosphorylation of all three human versions of Cdc25 is essential for cell cycle progression. Several phosphorylation sites have been mapped, suggesting the possibility that more than one kinase is involved in this regulation of Cdc25 by post-translational modification.

Cdc25 can be phosphorylated by its own substrate, cyclin-dependent kinases (Cdks). Cdc25C is phosphorylated and activated by Cdc2/cyclin B *in vitro*. *In*

vivo, this activation occurs at the G2/M transition, which initiates mitosis (Hoffmann et al., 1993; Izumi and Maller, 1993; Strausfeld et al., 1994). Cdc25A was later

Figure 1. Cdc25 is a dual-specificity protein phosphatase activating cyclin-dependent kinases (Cdk)

Cdks bind to cyclins and are phosphorylated on three residues. Thr160 phosphorylation (Pa, shown in green) activates the kinase. The phosphorylations on Thr14 and Tyr15 (Pi, shown in red) are inhibitory and removed by Cdc25, resulting in an active kinase. The kinases Wee1 and Myt1 are counter-acting Cdc25 and phosphorylate Tyr15 and Thr14, respectively.

Figure 2 Cdc25 and the cell cycle.

This scheme summarizes the regulation and function of human Cdc25A, B and C in the cell cycle, as described in the text. Green arrows indicate induction, (de)phosphorylation or activation, red lines inhibition. Proteins with a Ubi tag are degraded via the ubiquitin-dependent pathway. Approximate timing of events and activity of proteins is indicated by the brown dashed lines subdividing the cell cycle into G0/G1, S, and G2/M phases.

shown to be phosphorylated by Cdk2/cyclin E *in vitro* (Hoffmann et al., 1994). *In vivo*, hyperphosphorylation of Cdc25A occurs during the S-phase (Jinno et al., 1994). These results suggest regulation of Cdc25 via a self-amplifying feedback loop. Such a cooperative phenomenon has been cited to explain the sharp rise of Cdc2 kinase activity at the G2/M transition (Hoffmann et al., 1993; Izumi and Maller, 1993; Strausfeld et al., 1994).

In addition to Cdks, other kinases are implicated in Cdc25 phosphorylation, as well.

For example, the Raf1 kinase turned out to associate with Cdc25. Using double immunofluorescence microscopy, Cdc25A and B were found to co-localize with Raf1 and Ras at the cell membrane (Galaktionov et al., 1995), a process that is dependent on serum stimulation. Raf1 kinase phosphorylates Cdc25A and B *in vitro*, leading to an increase in phosphatase activity (Galaktionov et al., 1995).

In a two-hybrid screen experiment, Raf1 also was found to be associated with members of the 14-3-3 protein family, which in turn associated with Cdc25A and B (Conklin et al., 1995). 14-3-3 proteins have been implicated in a number of mitogenic signaling pathways, including the kinase cascade that contains Raf1 (Fantl et al., 1994; Freed et al., 1994).

Moreover, a role for 14-3-3 proteins in the regulation of Cdc25 was reported in connection with DNA damage sensing in cells. The response of cells to UV-induced DNA damage is multifaceted. It involves induction of cyclin-dependent kinase inhibitors, such as p21^{Cip1/Waf1}, as well as hyperphosphorylation of the Cdks, and ultimately leads to cell cycle arrest (Poon et al., 1996). Recently, a new pathway has been proposed that links the gene sensing DNA damage in the yeast *S. pombe*—Rad3—to Cdc25 activity (Furnari et al., 1997; Sanchez et al., 1997). Rad3 is related to the human ATM protein that is defective in *ataxia telangiectasia* patients, a rare genetic disorder whose varied symptoms include possibly a high risk of developing tumors (Xu and Baltimore, 1996).

DNA damage induces increased phosphorylation of the Chk1 kinase by a Rad3-dependent process. Cdc25 is potentially a direct target of Chk1, and Chk1's phosphorylation of a specific serine residue (Ser216 in human Cdc25C) results in binding of Cdc25 to 14-3-3 protein (Peng et al., 1997). It was proposed that 14-3-3 binding sequesters Cdc25C from functionally interacting with Cdc2, leading to a G2 arrest in the cell cycle. Regulation of Cdc25 by spatial sequestering rather than inhibition of the phosphatase activity seems to be the main effect of the phosphorylation of Cdc25 via the Chk1

kinase. The Chk1 phosphorylation site is conserved in Cdc25A and B, as well, suggesting that a similar regulatory mechanism is involved in other DNA damage checkpoints earlier in the cell cycle.

The role of the 14-3-3 proteins in connection with the Raf1 kinase is still unclear. One can speculate that 14-3-3 proteins act as docking sites—or adaptors—for both Cdc25 and Raf1, and that subsequent phosphorylation of Cdc25 by Raf1 leads to the release and activation of Cdc25. This example nicely illustrates the fine balance of counter-acting processes in cell cycle regulation. Furthermore, it identifies Cdc25C, and possibly Cdc25A and B, as *bona fide* checkpoint genes.

Yet other kinases have been reported to phosphorylate Cdc25 protein, suggesting that there are additional mechanisms for coordinating the regulation of cyclin-dependent kinases with various mitotic processes, such as chromosome segregation (Kumagai and Dunphy, 1996).

III. Other regulatory mechanisms

The level of Cdc25 protein is tightly regulated by both transcriptional and post-translational mechanisms (Ducommun et al., 1990; Moreno et al., 1990). In humans, Cdc25A is expressed early in the G1 phase of the cell cycle following serum stimulation of quiescent fibroblasts (Jinno et al., 1994). Cdc25 B is expressed closer to the G1/S transition, and Cdc25C is activated in G2 (Sadhu et al., 1990).

Recently, Galaktionov and colleagues observed that Cdc25 mRNA became more abundant following activation of the Myc proto-oncogene. They were able to show that Cdc25A, and possibly Cdc25B, are physiologically relevant and direct targets of c-Myc (Galaktionov et al., 1996). Their studies suggest furthermore that Cdc25 is a general mediator of Myc function. Therefore, Cdc25 is not only essential to normal cell proliferation but also for inducing Myc-dependent apoptosis.

Downregulation of Cdc25 was reported to be achieved by at least two different mechanisms in the cell: repression and ubiquitin-dependent degradation.

As an example for repression, consider TGF- β . Its effect on cyclin-dependent kinase activity has been extensively studied as a model anti-mitogenic response, in particular in connection with cyclin-dependent kinase inhibitors (CKIs). In a recent report, Iavarone et al. conclude that induction of the cyclin-dependent kinase inhibitor p15^{Ink4B} and downregulation of Cdc25A by TGF- β constitute two complementary mechanisms of inhibition of the cyclin D-dependent kinase (Iavarone and Massague, 1997). Their experiments indicate that Cdc25A

downregulation by TGF- β occurs at transcription; it remains to be determined whether Myc participates in this process.

Ubiquitin-dependent degradation of proteins is an important regulatory mechanism for all sorts of cellular processes (reviewed in Ciechanover, 1994) and has been found to play a key role in the degradation of the mitotic cyclins (Glutzer et al., 1991). In a study on Cdc25 degradation in *S.pombe*, Nefsky and Beach isolated a gene named Pub1, which encodes an E6-AP like protein (Nefsky and Beach, 1996). E6-AP belongs to a family of ubiquitin ligases, or E3s, which assist in transferring a ubiquitin molecule or a polyubiquitin chain to a target protein. Once the target protein is tagged with ubiquitin, it is rapidly degraded by the 26S proteasome. Cdc25 was ubiquitinated in a Pub1-dependent fashion, and loss of Pub1 function lead to elevated levels of Cdc25 protein and increased Cdc25 activity *in vivo*.

IV. Cooperation of Ras and Myc

The regulation of Cdk activity involves inhibitory small proteins (cyclin-dependent kinase inhibitors, or CKIs) from the Ink and the Waf1/Kip1/Cip1 families. Recent findings suggest, firstly, that the regulation of Cdc25 and the CKI proteins through Ras and Myc is tightly interconnected and, secondly, that the cooperation of active Ras and Myc leads to accumulation of G1 Cdk activity (Leone et al., 1997). Expression of Myc and Ras results in a loss of p27^{Kip1} protein (probably through ubiquitin-dependent proteolysis) and leads to increased Cdk2/cyclin E activity. At the same time, Cdc25A is induced by c-Myc and activated by Raf1, a downstream target of Ras. This leads to a synergistic effect in removing an inhibitory protein and inhibitory phosphorylations on Cdk2/cyclin E, culminating in induction of S-phase.

Interestingly, the competition between p21 and Cdc25 can be demonstrated directly in binding experiments. Saha et al. identified a consensus sequence in p21 and Cdc25 that is important for their binding to Cdk complexes (Saha et al., 1997). p21 protein directly competes with Cdc25A and *vice versa*, suggesting that the two proteins utilise similar docking sites on the Cdk/cyclin complexes.

V. Cdc25 and cancer

Cdc25A and B have oncogenic properties. In rodent cells, human Cdc25A and Cdc25B, but not Cdc25C, phosphatases cooperate with either an activated Ras allele or loss of Rb1 in oncogenic focus formation (Galaktionov et al., 1995). Such transformants are highly aneuploid, grow in soft agar, and form high-grade tumours in nude mice. Based upon these criteria, Cdc25A and B are *bona fide* cellular proto-oncogenes.

Indeed, Cdc25B mRNA is expressed at high levels in 32 percent of human primary breast cancers tested (Galaktionov et al., 1995). Similar findings have come from breast cancer studies on Cdc25 A (M. Loda et al., unpublished). Overexpression of Cdc25A and Cdc25B, but not Cdc25C, has also been reported in more than 50 percent of tested squamous cell carcinomas of the head and the neck (Gasparotto et al., 1997).

Given the tight connection between Cdc25 and the well-known oncogenes Ras and Myc, overexpression and activation of Cdc25 might be an important feature in cancer development, making Cdc25 an attractive target for future cancer therapy.

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