CDC25 phosphatases in cancer cells: key players? Good targets?

Rose Boutros, Valérie Lobjois* and Bernard Ducommun*‡*

Abstract | Cell division cycle 25 (CDC25) phosphatases regulate key transitions between cell cycle phases during normal cell division, and in the event of DNA damage they are key targets of the checkpoint machinery that ensures genetic stability. Taking only this into consideration, it is not surprising that CDC25 overexpression has been reported in a significant number of human cancers. However, in light of the significant body of evidence detailing the stringent complexity with which CDC25 activities are regulated, the significance of CDC25 overexpression in a subset of cancers and its association with poor prognosis are proving difficult to assess. We will focus on the roles of CDC25 phosphatases in both normal and abnormal cell proliferation, provide a critical assessment of the current data on CDC25 overexpression in cancer, and discuss both current and future therapeutic strategies for targeting CDC25 activity in cancer treatment.

Dual-specificity protein phosphatase
A phosphoprotein phosphatase that is able to hydrolyse the phosphate ester bond on both a tyrosine and a threonine or serine residue on the same protein.

Cyclin-dependent kinase
In association with their cyclin regulatory subunits, CDKs control progression through key cell cycle transitions.

**LBCMCP-CNRS UMR5088, IFR109 Institut d'Exploration Fonctionnelle des Génomes, University of Toulouse, 118 route de Narbonne, 31062 Toulouse, France.*
‡UA4124. CHU Purpan, Pavillon Lefebvre TSA 40031, 31059 Toulouse Cedex, France.
Correspondence to B.D.
e-mail: ducommun@cict.fr
doi:10.1038/nrc2169
Published online
14 June 2007

The cell division cycle 25 (CDC25) family of proteins are highly conserved dual specificity phosphatases that activate cyclin-dependent kinase (CDK) complexes, which in turn regulate progression through the cell division cycle. CDC25 phosphatases are also key components of the checkpoint pathways that become activated in the event of DNA damage. All cells are constantly subjected to stress, such as UV radiation or the production of free oxygen radicals, which can potentially cause DNA damage. Usually the cell responds by activating a relevant checkpoint mechanism, which causes cell-cycle arrest and mediates either repair of the damaged DNA or programmed cell death. However, when these pathways are defective the cell continues to divide and the DNA lesion is passed to daughter cells, resulting in a loss of genome integrity. Misregulation of CDC25 phosphatases, one of the essential components of checkpoint mechanisms, can therefore contribute to genomic instability. Their key roles in cell-cycle control and their abnormal expression in cancer cells make CDC25 phosphatases ideal targets for cancer therapy.

CDC25 phosphatases in cell-cycle control

CDC25 phosphatases are found in all eukaryotic organisms except plants[1]. In mammalian cells, three isoforms have been identified: CDC25A, CDC25B and CDC25C[2–4]. Orthologues of these isoforms have been found in *Xenopus laevis* (CDC25A and CDC25C) and in chicken (*Gallus gallus*; CDC25A and CDC25B)[5–7] (FIG. 1a). Among the different species, the catalytic domains of CDC25 proteins are quite conserved compared with the regulatory regions, which are far more diverse and further subjected to alternative splicing events that generate at least two variants for CDC25A[8] and five each for CDC25B[9,10] and CDC25C[8,11] (FIG. 1b). The non-catalytic domain dictates the intracellular localization and turnover of the phosphatases, but little is known about the specific function(s) of each isoform. Therefore, although there is only one CDC25 phosphatase in lower eukaryotes such as yeast[12], a potentially wide variety of CDC25 phosphatase activities exists in mammalian cells.

A common mode of action. CDK complexes are key regulators of cell-cycle progression, and are held inactive by the phosphorylation of two residues located within the ATP binding loop (Thr14 and Tyr15 of CDK1), by the WEE1 and MYT1 kinases[13]. When CDK activity becomes required for progression into the next cell-cycle phase, the dual specificity CDC25 phosphatases dephosphorylate these two residues, thereby activating the CDK–cyclin complex (FIG. 2a). One further activating phosphorylation (Thr161 of CDK1) by CDK-activating kinase (CAK) is required for their complete activation[14], although this phosphorylation does not seem to be regulated by cell signalling networks.

To date, CDK–cyclin complexes are the only known substrates for CDC25 phosphatases[15]. In mammalian cells, all three isoforms have been implicated in the

At a glance

- Cell division cycle 25 (CDC25) phosphatases are key regulators of the eukaryotic cell cycle. They are required to control cyclin-dependent kinase (CDK) dephosphorylation and activation in a strict spatio-temporal manner.
- CDC25A, B and C expression and activity is tightly regulated by many mechanisms, including alternative exon splicing, phosphorylation–dephosphorylation cycles, interactions with partners such as 14-3-3 proteins, intracellular localization and cell-cycle controlled degradation.
- CDC25 phosphatases are key targets of the checkpoint machinery activated in response to DNA damage. They are functionally inactivated or degraded to stop cell-cycle progression. CDC25B activity is required for checkpoint recovery.
- CDC25A and CDC25B overexpression are frequently found in many cancers, and are often associated with high-grade tumours and poor prognosis.
- The contribution of CDC25 phosphatases to tumorigenesis might be related to the genetic instability associated with the checkpoint-abrogating effect of their overexpression.
- Compounds that inhibit CDC25 phosphatase activities are currently being developed. The most potent quinonoid-based compounds identified so far are active on xenografted tumour models.

control of the G1–S and G2–M transitions by regulating the activities of CDK1 and CDK2. CDC25A mainly activates the CDK2–cyclin E and CDK2–cyclin A complexes during the G1–S transition[16–18], but also has a role in the G2–M transition[19,20] by activating CDK1–cyclin B complexes, which are thought to initiate chromosome condensation[19,21–23]. CDC25B and CDC25C are primarily required for entry into mitosis[24–26]. CDC25B is proposed to be responsible for the initial activation of CDK1–cyclin B at the centrosome during the G2–M transition[21,26,27], which is then followed by a complete activation of CDK1–cyclin B complexes by CDC25C in the nucleus at the onset of mitosis[28]. Knockdown experiments of CDC25B or CDC25C using antisense or interference RNA showed that these two phosphatases are also involved in the control of S-phase entry[29,30]. So, all three CDC25 phosphatases seem to function as key regulators of the G1–S and G2–M transitions and of mitosis, to spatially and temporally regulate their respective CDK substrates (FIG. 2b,c). The apparent functional redundancy that exists between CDC25A, B and C remains controversial. On the one hand, double knockout (*Cdc25b*$^{-/-}$;*Cdc25c*$^{-/-}$) mice develop normally, suggesting that CDC25A is capable of performing all CDC25 functions, and no *Cdc25a* knockout mouse model has been reported to date[31]. On the other hand, CDC25B has been shown to be essential for meiotic resumption in female mice[32], indicating that perhaps the redundancy between the different CDC25 isoforms is limited.

Cell-cycle regulation of CDC25 activity. In order to ensure timely progression through the various phases of the cell cycle, CDC25 phosphatase activities are themselves highly regulated by multiple mechanisms, including inhibitory and activating phosphorylations, changes in intracellular localization and interactions with partner proteins (FIG. 3). The phosphorylation of the N-terminal regulatory domains by several kinases, including the CDK–cyclin complexes themselves, have been reported to regulate the activities of the CDC25 phosphatases[23,33]. For example, a pool of CDC25B is phosphorylated and activated by Aurora A at the centrosome, where CDK1–cyclin B is initially activated, and this phosphorylation may locally participate in the control of the onset of mitosis[34–36] (FIGS 2c,3). CDC25B and C phosphatases are then activated by CDK1–cyclin B complex-dependent phosphorylation, leading to an irreversible auto-amplification loop that drives cells into mitosis[25,28,37]. Polo-like kinase 1 (PLK1) also phosphorylates and activates CDC25C leading to further amplification of CDK1–cyclin B activity in mitosis[38–42], and nuclear import of CDC25C was reported to be induced following the phosphorylation of serine residues within its nuclear export signal (NES) by the PLK1 and PLK3 kinases[23,43,44]. A similar auto-amplification mechanism has been shown for CDC25A by CDK2–cyclin E complexes at the G1–S transition[17].

By contrast, the phosphorylation of CDC25C by CHK1 and CTAK1 kinases during the normal cell cycle and after DNA damage, on a specific serine residue generates a binding site for the 14-3-3 family of proteins, leading to CDC25C sequestration in the cytoplasm during interphase and the inhibition of mitotic entry because of maintained inhibitory CDK1 phosphorylation[23,45]. Similarly, maternal embryonic leucine zipper kinase (MELK; also know as pEG3) and CHK1 kinases have been shown to phosphorylate CDC25B at the centrosome, thereby inhibiting mitotic entry[46–52]. CHK1 also controls the proper timing of mitotic onset by phosphorylating CDC25A, thereby creating a 14-3-3 binding site to prevent its interaction with CDK1–cyclin B[53].

Phosphorylation can also regulate CDC25 phosphatase stability. Although CDC25C protein levels remain fairly constant throughout the cell cycle[30], CDC25A and CDC25B protein levels have been reported to vary in a cell-cycle-dependent manner. CDC25A is predominantly expressed in G1 and stabilized in mitosis through CDK1–cyclin B-mediated phosphorylation[18,22]. At the end of mitosis, CDC25A levels rapidly decrease owing to its degradation, mediated by anaphase promoting complex/cyclosome (APC/C)-dependent ubiquitylation[54]. In S and G2 phases, CDC25A is targeted for proteasome-mediated degradation by the SKP1–CUL1–Fbox (SCF)$^{\beta TRCP}$, a component of the SCF ubiquitin ligase complex. SCF$^{\beta TRCP}$-dependent degradation of CDC25A in S-phase requires phosphorylation of serine clusters by CHK1 and/or CHK2 and another as yet unknown kinase[23,55–58]. CDC25B levels begin to increase from mid S-phase, peak during the G2–M transition, and then rapidly decrease[26,30,164]. CDC25B is degraded by the proteasome pathways, and this degradation is dependent on phosphorylation by CDK1–cyclin A and involves the binding of SCF$^{\beta TRCP}$ (REFS 59,60).

Multiple checkpoint pathways are activated in response to DNA damage or environmental insults in order to block the G1–S or G2–M transitions or S-phase progression (FIG. 4a). Regardless of the initial insult, the net result is an inhibition of CDK–cyclin complexes in order to stop cell-cycle progression. Incomplete DNA replication or DNA damage, such as

Checkpoint
Checkpoint mechanisms control the order and timing of crucial cell-cycle transitions and ensure that crucial requirements (DNA integrity, chromosome partitioning) are met, for the maintenance of the genome.

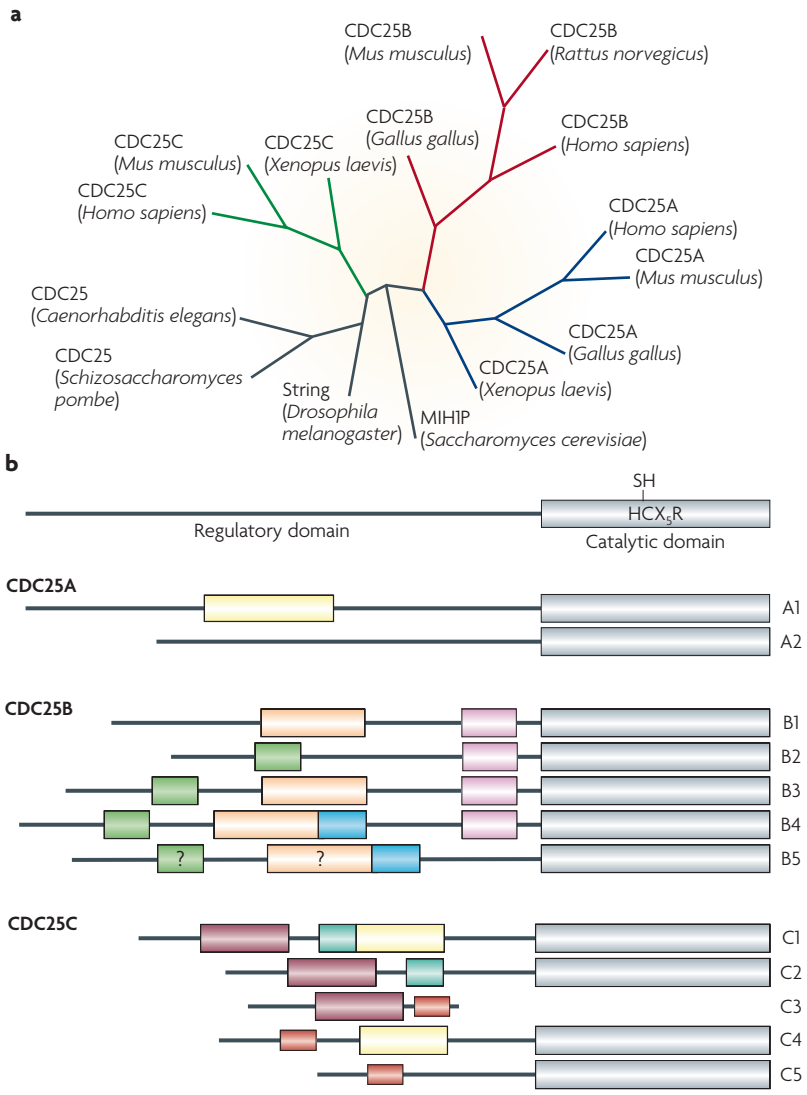


Figure 1 | **The CDC25 family from an evolutionary perspective. a** | Phylogenetic analyses of the amino acid sequences of cell division cycle 25 (CDC25) phosphatases. Full length sequences were aligned using ClustalW[160]. An unrooted tree was constructed using the PHYLIP package[161]. Bootstrapped sets of multiple sequence alignments and distance matrices (100 replicates) were created using Seqboot and Protdist, respectively. Neighbour was used to construct a bootstrapped set of phylogenetic trees (100 replicates), and the consensus tree shown was constructed using the Consense program. **b** | The N-terminal regulatory domains of human CDC25A, CDC25B and CDC25C are subject to alternative exon splicing (coloured boxes), generating multiple isoforms. For CDC25A, one exon is skipped in CDC25A2 compared with CDC25A1. In the CDC25B splice variants, four exon-encoded domains are differentially present in each isoform. The different variants of CDC25C result from the differential splicing of three exons. The question marks indicate that the presence of these two domains has not been confirmed in CDC25B5. In CDC25C4 and CDC25C5, the alternative splicing causes a translational frameshift, introducing new amino acids (brown boxes). In CDC25C3, the deletion also leads to a frameshift that gives rise to a truncated CDC25C protein that lacks phosphatase activity. The highly conserved catalytic domain of CDC25A, B and C is shown in grey. The catalytic cysteine residue is also indicated. Figure not drawn to scale.

that caused by ionizing radiation or UV light, activate the DNA damage checkpoint through the ATM (ataxia-telangiectasia mutated) and ATR (ataxia-telangiectasia and Rad3-related) kinases. ATM is primarily activated in response to double strand DNA breaks, whereas ATR seems to be mainly activated by regions of single-stranded DNA, although some redundancy and cooperation between the ATM and ATR signalling pathways does exist[61]. Consistent with this, ATR and ATM kinases phosphorylate and activate the checkpoint kinases CHK1 and CHK2 (REF. 62), which have several effector substrates, including the CDC25 phosphatases. CHK1 and CHK2 mediate the inhibition and/or degradation of the CDC25 phosphatases. CHK1 mediates S and G2 phase arrest through CDC25A degradation in response to chemically-, UV- or ionizing radiation-induced DNA damage[20,23,63–65]. Sorensen *et al.*[66] have further shown that after exposure to ionizing radiation, the hyper-phosphorylation of CDC25A by both CHK1 and CHK2 promotes the accelerated turnover of CDC25A, which has been shown to be mediated by $SCF^{\beta TRCP}$ (REFS 33,56). CDC25C is also phosphorylated by CHK1 and/or CHK2 kinases in response to DNA damage and ATM and/or ATR activation, leading to subsequent binding of 14-3-3 proteins and cytoplasmic sequestration of CDC25C away from CDK1–cyclin B[49,67,68] (FIG. 4b). In addition to the inhibition of CDC25 phosphatases, CHK1 activation also increases WEE1 activity to further reinforce the inhibition of CDK–cyclin complexes[69,70].

The p38 mitogen-activated protein kinase (MAPK) pathway, a stress-activated signalling pathway that delays the G2–M transition in response to cellular stimuli such as osmotic stress or UV irradiation, has also been shown to regulate the CDC25s[33,71]. p38 and its downstream target, mitogen-activated protein kinase-activated protein kinase 2 (MAPKAPK2) were initially shown to phosphorylate CDC25B and CDC25C *in vitro* and *in vivo* after UV-induced DNA damage, or induce CDC25A degradation after cisplatin treatment[72–75]. These data were recently extended to include the demonstration that this pathway also functions as an alternative checkpoint response to UV-induced DNA damage in tumour cells in which p53 is absent[75]. Surprisingly, the p38–MAPKAPK2 pathway was shown to be directly activated by DNA-damaging chemotherapeutic agents, and to be essential for the resistance of p53-deficient (but not p53-proficient) tumour cells to chemotherapy-mediated cell death[75]. Finally, CDC25B (but not CDC25A or CDC25C), is required along with PLK1 for recovery from a G2 DNA-damage-induced arrest and entry into mitosis when DNA damage has been dealt with[76]. In line with this observation, CDC25B protein levels have been reported to increase after DNA damage by UV radiation, ionizing radiation or genotoxic agents, and increased CDC25B expression leads to the bypass of genotoxic-induced G2/M checkpoint arrest[77,78] (FIG. 4c).

Thus, the activities of CDC25 phosphatases are highly regulated, both during the normal cell division cycle and in response to checkpoint activation, in order to ensure that a correct level of CDK–cyclin activity is maintained. This, in turn, controls the progression of the cell through the division cycle in an orderly manner, and ensures the maintenance of genomic stability. Therefore, the misregulation of any of these control mechanisms could result in the acquisition of genetic mutations and so contribute to tumorigenesis.

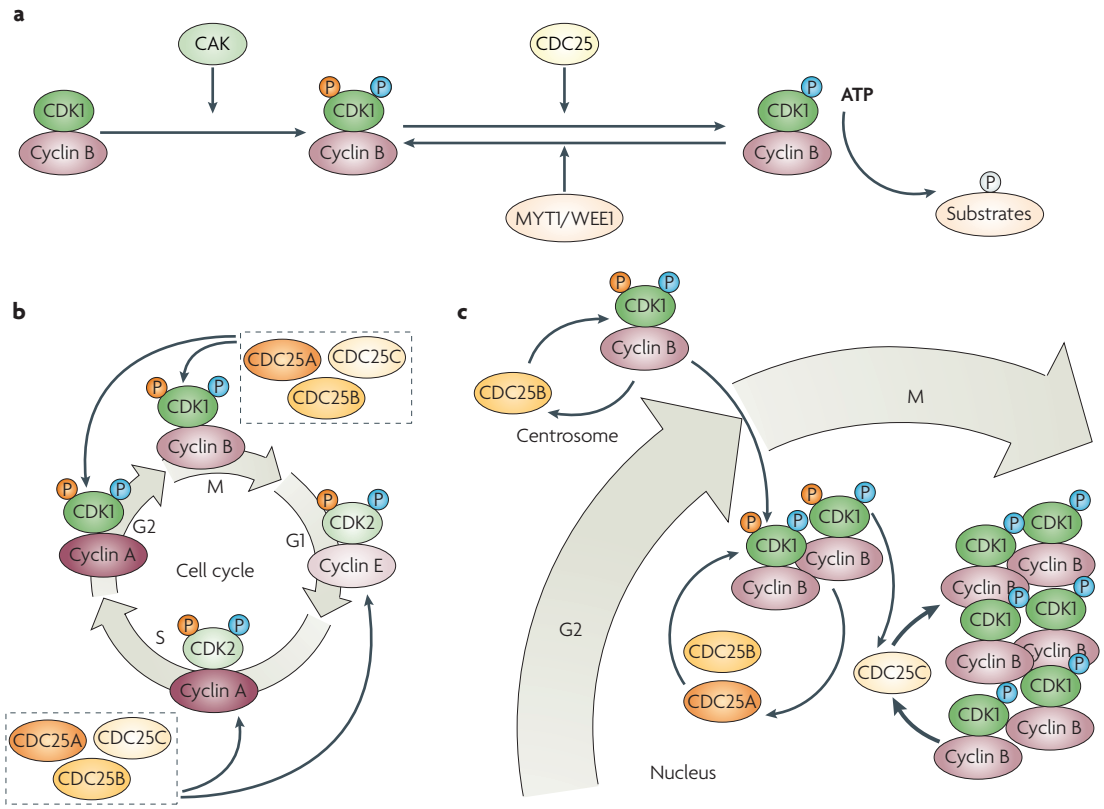


Figure 2 | **CDC25 phosphatases control key cell cycle transitions. a** | Cell division cycle 25 (CDC25) phosphatases dephosphorylate and activate cyclin-dependent kinase (CDK)–cyclin complexes, thus allowing catalysis and substrate phosphorylation. WEE1 and MYT1 kinases phosphorylate CDK on tyrosine 15 and threonine 14 of CDK1. Phosphorylation by the CDK-activating kinase (CAK) is required for further activation of CDK–cyclin complexes. For simplicity, an orange P represents T14 and Y15 phosphorylation by WEE1 and MYT1, a blue P represents T161 phosphorylation by CAK. **b** | Although initial studies suggested a specific role for each CDC25 phosphatase at defined stages of the cell cycle, the current model is that CDC25A, B and C are all involved in phosphorylating CDK–cyclin complexes, such as CDK2–cyclin E at the G1–S transition or CDK1–cyclin B at the entry into mitosis. **c** | CDC25A, B and C control entry and progression into mitosis. CDC25B is thought to be responsible for the initial activation of CDK1–cyclin B at the centrosome that contributes to microtubule network reorganization and mitotic spindle assembly. Nuclear translocation leads to an auto-amplification process (bold arrows) of CDC25s that then fire the bulk of CDK1–cyclin B complexes and trigger mitosis.

CDC25 in cancer

As detailed above, CDC25 phosphatases must be tightly regulated throughout the cell division cycle to maintain the precise spatial and temporal level of CDK–cyclin activities. In addition, CDC25 phosphatases must be inactivated in response to checkpoint activation to stop cell cycle progression and allow the cell time to either repair the DNA or initiate apoptosis. Misregulation of these processes can contribute to genomic instability. Consistent with this, CDC25 overexpression has been reported in various human cancers, often in correlation with more aggressive disease and poor prognosis.

CDC25 overexpression: a recurring theme in cancer. The first report to link CDC25 isoform expression and cancer came from the study by Nagata *et al.*[4] in which *CDC25Hu2* (now *CDC25B*) mRNA was found to be overexpressed (relative to β-actin and *CDC25Hu1* (now *CDC25C*) mRNAs) in cancer cell lines and SV40-transformed fibroblasts. Since then, CDC25 phosphatases, particularly the CDC25A and CDC25B isoforms, have been reported to be overexpressed in primary tissue samples from various human cancers, including breast[79–82], ovarian[83], prostate[84,85], lung[86,87], colorectal[88,89,90], oesophageal[91–95], thyroid[96–98], laryngeal[99], hepatocellular[100], gastric[101], pancreatic[102], endometrial[103,104], head and neck cancer[105], neuroblastoma[106], glioma[107] and non-Hodgkin lymphoma[108–111]. This is shown in FIG. 5, which (for the purpose of simplicity) summarizes only those studies in which CDC25 isoforms have been reported to be overexpressed at the protein level.

A critical assessment of the data on CDC25 overexpression in cancer shows, first and foremost, that in specific cancer subtypes there is a correlation between CDC25 level and specific clinicopathological features, such as higher grade tumours and poor disease-free survival. However, the fact that in many tissues CDC25 proteins are expressed at levels below the limit of detection by currently available methods, has significantly limited attempts to assess their role in cancer. As a result, differing techniques have been applied to analyse CDC25 RNA or protein expression, in different patient cohorts

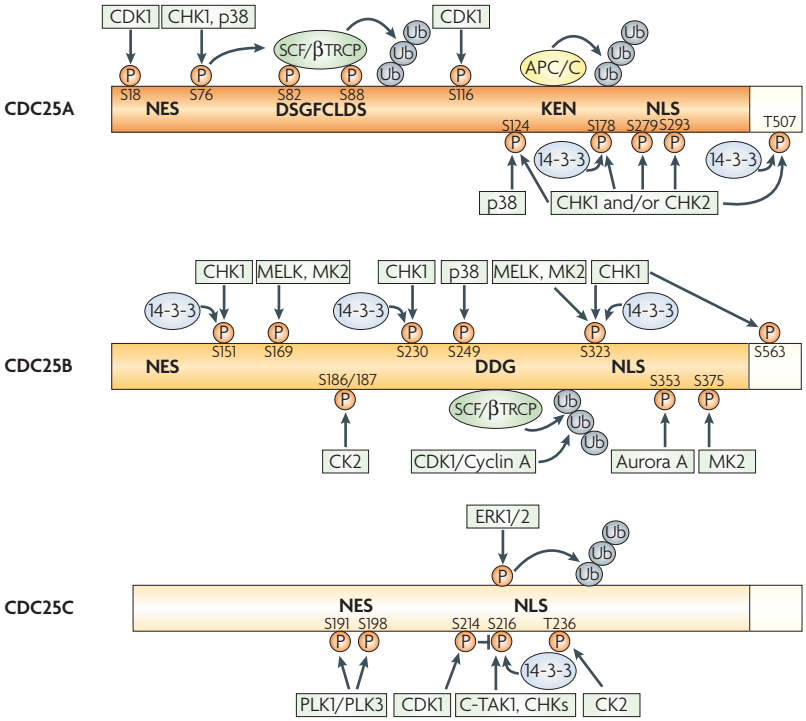


Figure 3 | **Multiple key phosphorylation events regulate CDC25 phosphatases.** A schematic view of our current knowledge of the phosphorylation sites for cell division cycle 25 (CDC25) phosphatases. Most of these phosphorylations are located within the N-terminal regulatory domain of the proteins (C-terminal catalytic domains are shown as cream boxes; not to scale). CDC25A, CDC25B and CDC25C are phosphorylated by multiple kinases that regulate their activity, interactions with 14-3-3 proteins, and intracellular localizations by the modulation of nuclear export sequence (NES) and nuclear localization signal (NLS). For simplicity, many additional putative cyclin-dependent kinase (CDK)–cyclin phosphorylation sites are not depicted here. Degradation by the $SCF^{\beta TRCP}$ or by the APC/C–ubiquitin (Ub)-dependent degradation pathways is also controlled by the indicated phosphorylation events.

(as reviewed by Rudolph *et al.*[15]), thereby limiting the extent to which data from multiple studies can be compared. One such limitation is the lack of correlation between RNA and protein levels reported, and this is most obvious in the studies in which both parameters were assessed in the same patient cohorts[87,88,106]. For example, *CDC25B* mRNA was reported to be overexpressed in 80% of neuroblastomas, whereas protein overexpression was detected in only 5% of the same samples[106]. Such inconsistencies have, in turn, contributed to contradictory reports on the utility of CDC25 overexpression as a prognostic indicator in specific cancer subtypes. For example, in breast cancer, CDC25B mRNA and protein were reported to be overexpressed at rates of 32%[82] and 57%[96] respectively, and although CDC25B overexpression was found to be associated with higher grade tumours in one study[82], it was associated with lower grade tumours in the other[96]. Similarly, CDC25A transcript and protein overexpression were reported in 52%[79] and 70%[96] of patients with breast cancer, respectively, and although *CDC25A* mRNA overexpression was reported to be associated with poor prognosis in one study[79], no association between CDC25A protein overexpression and clinicopathological features was found in the other[96]. In addition, one cannot be sure that in each case the overexpression of CDC25 levels translates to increased activity towards CDK–cyclin substrates, further limiting the extent to which we can compare between studies. Therefore, although it is clear that both CDC25A and CDC25B are commonly overexpressed in breast carcinomas, the significance of this remains somewhat clouded by the contradictory issues described herein. Similar principles also apply to CDC25 overexpression in other cancers.

As shown in FIG. 5, CDC25 overexpression within each cancer subtype tends to occur in an isoform-specific manner, and the overexpression of multiple isoforms in the same cancer subtypes probably occur through independent pathways. For example, *CDC25A* and *CDC25B* transcripts were reported to be overexpressed in 60% and 45% of lung cancers, respectively, and although both were associated with poorly differentiated tumours and/or reduced survival[86,87], the incidence of overexpression of each isoform was found to be independent of the other, as only 30% of tumours overexpressed both *CDC25A* and *CDC25B* compared with 45% that overexpressed one or the other[87]. Although both CDC25A and CDC25B were significantly overexpressed in colorectal cancer, only CDC25B levels were found to be of prognostic value in several independent studies[88–90]. In fact, high expression of CDC25B is more frequently associated with higher grade or later stage tumours[82,84,89,90,98,99,101,103,107–110,112] than that of CDC25A[109,112], and although both CDC25A and CDC25B overexpression have been associated with shorter disease-free survival, these were reported in breast[79], oesophageal[112] and hepatocellular[100] carcinomas for CDC25A and in lung[86] and thyroid[98] carcinomas and gliomas[107] for CDC25B.

Several other factors support the notion that independent pathways could be involved in the overexpression of CDC25 isoforms. For example, only CDC25A overexpression shows an inverse association with the p27 tumour suppressor, in that increased *CDC25A* mRNA along with decreased p27 mRNA or protein levels have been associated with aggressive disease and poor prognosis in non-Hodgkin lymphoma[110] and breast carcinoma[79]. Similarly, only CDC25B overexpression has been associated with increased sensitivity to radiotherapy in oesophageal cancer[92,94,95]. Finally, although the overexpression of CDC25C has been reported in a limited number of studies, its rate of overexpression in tumours compared with normal tissues is much lower than those of CDC25A and CDC25B[85,89,104,109].

In addition to differences between isoforms, examination of splice variants (FIG. 1b) of individual CDC25 isoforms by reverse transcriptase PCR (RT-PCR) in a few studies have revealed that some are more commonly involved in cancer than others. For example, *CDC25B3* rather than *CDC25B1* or *CDC25B2* splice variants were found to be overexpressed in most pancreatic cancers[102], whereas overexpression of the *CDC25B2* splice variant correlates with tumour grade in colorectal cancer[90] and increased tumour aggression in non-Hodgkin lymphoma[109]. The *CDC25C5* splice variant was found to have increased mRNA levels in 50% of prostate cancers compared with 17% normal prostate tissue[85].

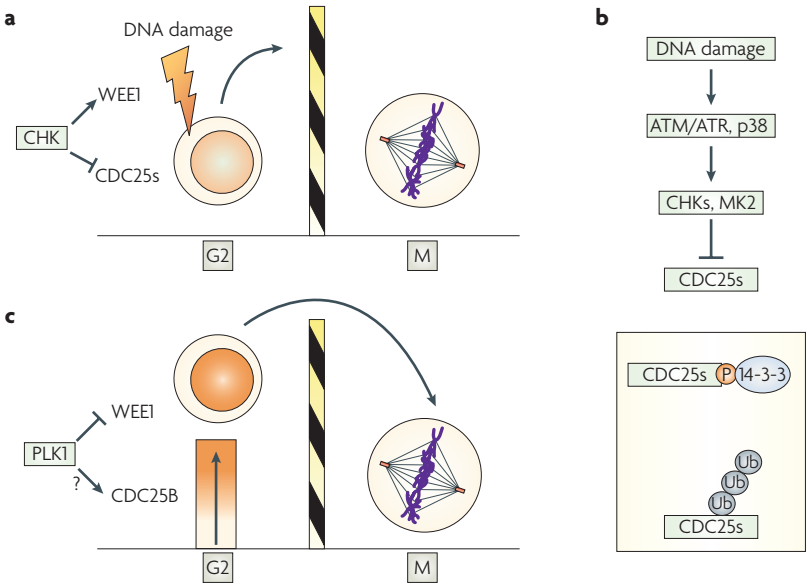


Figure 4 | **The checkpoint hurdle to enter mitosis. a**, **b** | Upon DNA damage, cell division cycle 25 (CDC25) proteins are inhibited through various mechanisms, including checkpoint kinase-dependent degradation or cytoplasmic sequestration through 14-3-3 binding (see REF. 23 for a review). The inhibitory kinases WEE1 and MYT1 are activated by checkpoint kinases. CDK–cyclin complexes are in turn maintained in their inactive state and the cell remains arrested in G2 phase. **c** | CDC25B level is central to the control of entry into mitosis after DNA damage. Together with PLK1 activity (which is required for WEE1 inhibition), CDC25B accumulation through a mechanism that is still unclear[78] is required to allow entry into mitosis when damage has been repaired[162]. Increased levels of CDC25B protein found in cancer might therefore facilitate checkpoint exit and increase genomic instability[77].

The contradictory nature of the mRNA and protein data reported in many instances might reflect, at least in part, some of the problems associated with the inability to determine the exact splice variant composition. But, with the development of specific tools, closer examination of the expression of each splice variant derived from each *CDC25* gene in cancer (particularly with respect to clinicopathological features), and in combination with current studies on the specific roles of each splice variant during the normal cell division cycle, is likely to provide powerful prognostic and perhaps therapeutic tools for individual cancer subtypes in the future.

What causes CDC25 overexpression? The mechanism(s) by which CDC25 isoforms become deregulated during tumorigenesis remains unclear. There is currently no evidence that CDC25 overexpression results from gene amplification or rearrangements or any other specific genetic mutations that may be responsible for deregulating CDC25 phosphatase activities in cancer. In addition, the lack of significant correlation between transcript and protein levels suggests that the CDC25 phosphatases can become misregulated at any stage between transcription and translation, even at the post-translational level.

At the level of transcription, both *CDC25A* and *CDC25B* were reported to be direct transcriptional targets of the MYC proto-oncogene in serum-starved fibroblast cell lines[113]. Activation of MYC resulted in increased *CDC25A* and *CDC25B* mRNA levels, which correlated with an increase in protein expression[113]. Both *CDC25A* and *CDC25B* genes were further shown to contain MYC/MAX binding sites, and have been proposed to cooperate with MYC in cell-cycle regulation[113]. Since this initial report in cultured cells, statistically significant correlations have been reported between increased CDC25A and MYC expression in breast cancer[114], and increased *CDC25B* and *MYC* mRNA levels in non-Hodgkin lymphoma[108] and lung carcinoma[86] or *NMYC* mRNA levels in neuroblastoma[106]. *CDC25A* has also been reported to be a transcriptional target of the E2F–RB1 (retinoblastoma 1) pathway[115,116], and its activity was shown to be essential for the efficient S-phase-inducing capacity of serum-starved cells transformed by E2F1 (REF. 115).

CDC25 transcript levels are also increased in response to virally induced cellular transformation[4,117]. *CDC25B* mRNA was highly elevated in fibroblast cells transformed by SV40 or by the E6 or E7 papilloma virus transforming proteins, all of which cause chromosomal aberrations and cell transformation[4]. Similarly, *CDC25A* mRNA was found to be elevated in quiescent human fibroblasts infected with the E1A adenovirus protein, and this was accompanied by a rapid increase in CDC25A phosphatase activity[117]. These data therefore suggest that *CDC25* promoters are specifically targeted by activated transcription factors and transforming viruses during the cellular transformation process.

At the post-translational level, the half-life of CDC25A protein in breast cancer cell lines that overexpress CDC25A was found to be increased compared with cell lines that express lower levels[118]. Thus, the increased stability of CDC25A protein, rather than an increase in transcripts, was proposed to be responsible for the increase in CDC25A protein and phosphatase activity in breast cancer cell lines[118]. Mutants of ATR or microcephalin, a tumour suppressor that functions downstream of ATR, were also shown to result in increased CDC25A stability in both unperturbed cell division and in response to UV radiation[119]. Given the role of the proteasome pathway in regulating the turnover of both CDC25A and CDC25B proteins (as discussed earlier), it is very possible that changes in the regulators of CDC25 stability could contribute to the increased expression of CDC25 proteins seen in many cancers. In support of this idea, mutations resulting in decreased βTRCP expression have been reported in several cancers, including prostate[120], lung[121] and gastric[122] carcinomas. In lung cancer cell lines, small interfering RNA (siRNA) inhibition of *βTRCP* was further shown to increase the stability of CDC25A[121], although this evidence was indirect, and requires further examination. Similarly, the CHK kinases (but more frequently CHK2 than CHK1) have been shown to be mutated and/or downregulated in several hereditary cancers, such as breast and colon tumours and sporadic cancers, including carcinomas of the breast, lung, ovary, colon, stomach and endometrium[123], brain tumours[124] and lymphomas, in which CHK1 was reported to be specifically downregulated at both the mRNA and protein levels in aggressive non-Hodgkin lymphoma[125]. However, similar to βTRCP, direct evidence for CHK mutations increasing

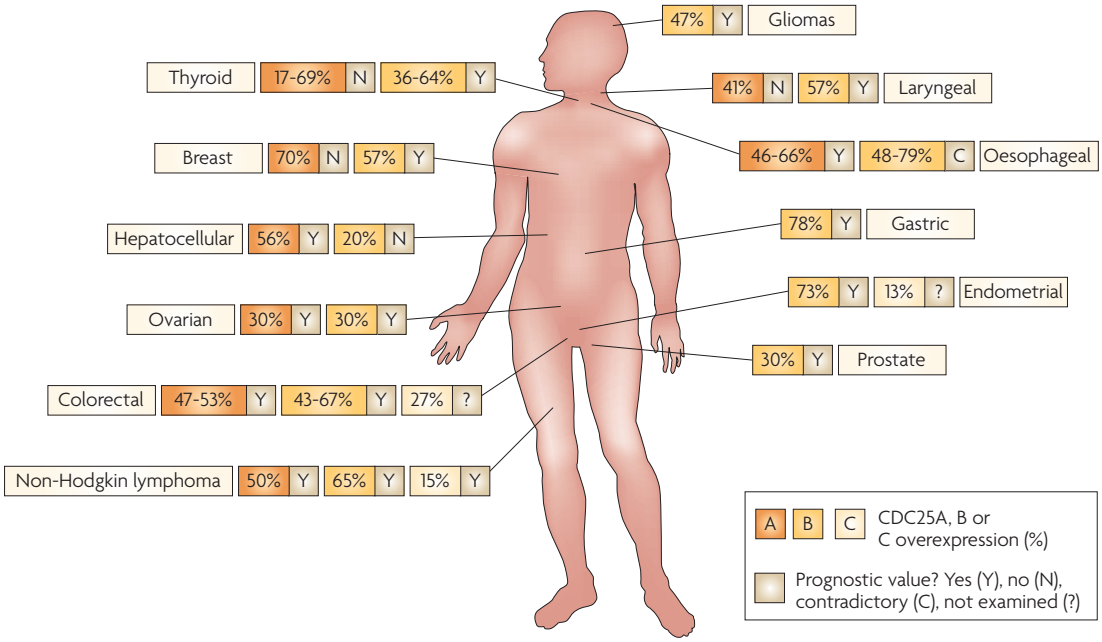


Figure 5 | **Overexpression of CDC25 protein in human cancers.** Cell division cycle 25 (CDC25) proteins reported to be overexpressed in primary tumour samples from patients with breast[96], prostate[84], ovarian[163], endometrial[104], colorectal[88,89], oesophageal[91,92,94,112], thyroid[96–98], laryngeal[99], gastric[101] and hepatocellular cancers[100], glioma[107], neuroblastoma[106] or non-Hodgkin lymphoma[109]. Percentages of tumours in which CDC25A, CDC25B or CDC25C proteins are overexpressed are indicated, or in the case of overexpression reported in more than one study, a range of percentages is given. The cut-off mark for inclusion was overexpression in >10% patients. Whether (Y) or not (N) CDC25 overexpression was linked to clinicopathological features, including tumour grade or stage, metastases, depth of invasion, residual or recurrent disease, or disease-free survival is marked in yellow boxes beside percentages. Cases for which several studies reported contradictory prognostic values are marked by a (C), and studies in which clinicopathological features were not assessed are indicated by an (?).

CDC25 stability is currently lacking. Finally, it is also likely that other, currently unidentified, mechanisms contribute to CDC25 overexpression.

How does CDC25 overexpression contribute to tumorigenesis? Several lines of evidence indicate that although overexpression of CDC25 can contribute to cancer, it is insufficient to cause it. *In vitro*, the co-expression of CDC25A or CDC25B cooperates with either oncogenic HRAS or the loss of RB1 to transform mouse embryonic fibroblasts, which were then capable of forming high-grade tumours *in vivo*[82]. Similarly, although the transgenic expression of CDC25A alone resulted in alveolar hyperplasia in the mammary tissue of mice but not spontaneous tumour growth, CDC25A overexpression in MMTV–v-Ha-*ras* or MMTV–c-*neu* transgenic mice significantly accelerated tumour growth and induced miscoordinated cell proliferation and multiple chromosomal aberrations[126]. Furthermore, the targeting of CDC25B overexpression to the mammary glands of mice resulted in the increased proliferation of mammary epithelial cells and hyperplasia[127], but required additional challenge with the DMBA (9,10-dimethyl-1,2-benzanthracene) carcinogen to induce tumour growth[128].

As discussed earlier, the transitions between each cell-cycle phase must be strictly regulated in a spatio-temporal manner to maintain genomic stability. One could therefore imagine that the overexpression of either CDC25A or CDC25B, provided that it was accompanied by increased phosphatase activity, could result in the over-activation of its target CDK–cyclin complexes, thereby pushing the cell through the cell cycle transitions in an untimely manner and acquiring genetic aberrations as a result. This is supported by experimental evidence in which the overexpression of CDC25B (but not CDC25C) was shown to rapidly push S or G2 phase cells into mitosis with incompletely replicated DNA[129], and overexpression of CDC25A accelerates entry into S phase[16,130] or induces mitotic events[19]. Conversely, the microinjection of antibodies against CDC25B or CDC25C, or transfection with inactive mutants, causes a G2 arrest[24–26], whereas those against CDC25A cause G1 arrest[17,18].

Inappropriate progression through the cell division cycle also requires the circumvention of checkpoint mechanisms. As outlined above, DNA damage results in checkpoint activation and cell-cycle arrest, during which time the cell either repairs its damaged DNA or undergoes apoptosis. As CDC25 proteins are targeted for degradation or inactivation in response to checkpoint activation, it is possible that the presence of abundant levels of CDC25 proteins, which cannot be either completely degraded by the proteasome system or efficiently sequestered away from their targets by 14-3-3s, could potentially allow the continued activation of CDK–cyclin complexes, even in arrested cells, and eventually push

the cell through the checkpoint barrier (as illustrated in FIG. 4c). This is supported by experimental evidence showing that increased CDC25B expression results in a premature exit from genotoxic stress-induced checkpoint arrest[77], and that CDC25B is essential for resuming the cell cycle after DNA damage-induced checkpoint arrest[76]. Similar results were obtained for CDC25A, as its overexpression abrogates the S-phase checkpoint, and causes radioresistant DNA synthesis[131].

Current data from human cancers suggest that CDC25 overexpression could probably occur at different steps along the tumorigenic pathway and contribute to the progression of the disease by causing genomic instability through the deregulation of the cell division cycle and/or cooperation with other oncogenes important in normal signalling pathways. CDC25 phosphatases, in providing a direct link between mitogenic signalling and the cell division cycle, are therefore ideally situated to facilitate cell transformation, and this in turn makes them ideal targets for a new anticancer therapy.

CDC25 and anticancer therapy

The crucial role played by CDKs in the control of the cell cycle make them attractive pharmacological targets for the development of antiproliferative cancer drugs (see REFS 132,133 for comprehensive reviews). For several years, various strategies have been proposed to directly or indirectly inhibit the activities of these enzymes. Direct inhibition is based on the use of competitive ATP analogues with well known compounds, such as Flavopiridol and Roscovitine, both of which are currently in phase I and II clinical trials on patients with various types of relapsed or refractory tumours, either alone or in association with currently used chemotherapeutic agents (see REFS 134,135 for examples). As activators of CDKs, CDC25 phosphatases are obvious candidates for the development of original approaches to indirectly inhibit CDK kinases and their effects on cell-cycle control.

Main families of compounds identified so far. The identified CDC25 inhibitory compounds, which inhibit all three CDC25 isoforms, belong to various groups including quinonoids, phosphate surrogates and electrophilic entities. Para-quinonoid compounds derived from vitamin K, which are the most numerous and active, probably act through the irreversible oxidation of the cysteine residue in the catalytic domain (CX_5R) (FIG. 1b) of CDC25 phosphatases into a sulphonic acid (Cys-SO_3-)[15,136]. The most potent compounds (TABLE 1) in this family are NSC663284 (REF. 137) and JUN1111 (REF. 138), which were characterized by the J. Lazo and P. Wipf group (University of Pittsburgh, USA), natural origin compounds such as adociaquinone B (a marine sponge extract derivative)[139], and compounds developed by the IPSEN pharmaceutical group in collaboration with our laboratory, such as BN82685 (REF. 140) and IRC-083864 (REF. 141), the most potent CDC25 inhibitory compound identified to date. Other inhibitors of CDC25 have been reported from non-quinonoid compound families, such as H32 (REF. 142), or phosphomimetic moieties such as dysidiolyde and its analogues[143], or compounds such as KR61170, which is also a potent protein tyrosine phosphatase 1B (PTP1B) inhibitor[144,145] thought to interfere with the arginine residue within the catalytic domain of CDC25 proteins.

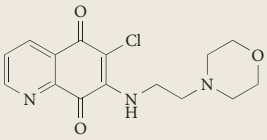
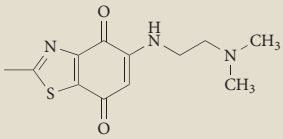
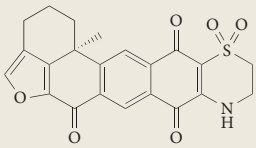
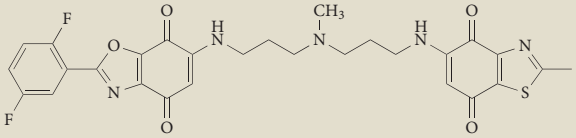
A limited set of representative compounds displaying low IC_{50} values (the half-maximal inhibitory concentration), compatible with drugability, is shown in TABLE 1. As indicated, the *in vitro* inhibitory activities of these compounds on the three CDC25 isoforms ranges from sub-μM to 20 nM for the most active compound. Most of these compounds have inhibitory activities on the proliferation of tumour cells at concentrations again ranging from μM to 23 nM for the ICR-083864 compound on the prostate cell line LNCaP, in culture. Detailed investigations have shown that these compounds arrest the cell cycle in G1, S and at G2/M phases or in one specific cell-cycle phase, depending on the cell type and the concentrations used (see REFS 140,146 for examples). In several instances, pro-apoptotic effects were also reported[147,148].

The specificities of CDC25 inhibitors have been examined *in vitro* against a few of the other members of the dual specificity phosphatase family, such as MKP1 (mitogen-activated protein kinase phosphatase 1) and VHR (vaccinia H1 related), and have been consistently found to be in favour of CDC25s. However, there are a few compounds that can also inhibit other phosphatases, such as PTP1B (KR61170 (REF. 145)). Experimental models based on growth inhibition assays in yeast or functional assays in cancer cells have been developed and used to validate the CDC25 selectivity of some of these compounds[140,149]. So far, very few CDC25 inhibitory compounds have been shown to inhibit tumour growth in xenografted nude mice. Published data are limited to the BN82002 (REF. 150) and BN82685 (REF. 140) compounds, both of which impair the growth of MiaPaCa human pancreatic carcinoma cells. The BN82685 compound was further reported to retain its activity when taken orally. The IRC-083864 compound is also active *in vivo* when taken orally on LNCaP xenografted tumours (G. Prévost, unpublished observations). The PM20 compound, although less active *in vitro*, has also been shown to be active *in vivo* on JM-1 hepatoma tumours when given intraperitoneally[151].

So, the number of potent inhibitors of CDC25 phosphatases that efficiently inhibit the proliferation of cancer cells and that are active *in vivo* on xenografted human tumours is still limited, and is restricted to compounds with quinonoid-based structures. Therefore, the development of inhibitors of transient protein–protein interactions might provide alternative strategies for a cancer therapy-based inhibition of CDC25 (REF. 152).

Is CDC25 inhibition alone sufficient to kill tumour cells? Conceptually, the inhibition of CDC25 addresses the question of its theoretical cytostatic effect. It is well known, since the founding work performed using thermosensitive mutants in yeast, that CDC25 inhibition results in a reversible cell-cycle arrest. Theoretically, given the fact that CDC25 proteins have short half-lives, one could expect that cells treated with a CDC25-inhibitory compound and arrested during a specific transition, should resume cell-cycle progression when the inhibitor is withdrawn. However, as observed in various models, unscheduled or

Table 1 | **CDC25 inhibitory compounds**

Compound	Chemical class	IC_{50} *in vitro*	IC_{50} cell lines	Active *in vivo*	Refs
NSC663284 	Quinolinedione	0.2 – 0.9 μM	0.2 μM (MDAMB435)	?	137, 138
BN82685 	Heterocyclic quinone	0.17 – 0.25 μM	0.1 μM (MiaPaCa2)	YES (Xenografted MiaPaCa2)	140
Adociaquinone B 	Naphthoquinone analogue	0.07 μM	ND	?	139
IRC083864 	Heterocyclic quinone	0.02 μM	0.02 μM (LNCaP)	YES (Xenografted LNCaP)	141

Most potent compounds (and formulae) identified so far that show inhibitory characteristics (IC_{50} values *in vitro* and *in cellulo*). *In vivo* data refers to the evaluation of the antiproliferative activity evaluation of the compounds on xenografted tumours in nude mice. The (?) stand for data not available. Important references are indicated.

partial escape from this arrest, as well as prolonged cell-cycle arrest, might ultimately be deleterious, leading the cell to enter an apoptotic programme. Nevertheless, this raises the question of whether it is necessary to associate CDC25 inhibitors with other currently used chemotherapeutic compounds. In line with this discussion, we have recently reported that the association between the CDC25 inhibitor BN82685 and the microtubule-targeting agent paclitaxel may have potent therapeutic potential[153]. Indeed, we have shown that BN82685 treatment resulted in alterations in microtubule dynamics that are likely to be mediated by an effect on CDK activity. Furthermore, we have found that the association of paclitaxel with CDC25 inhibitors results in an additive effect on cancer cells[153]. Cancers currently treated with paclitaxel, and in which CDC25 levels were found to be increased, such as ovarian tumours, might benefit from this type of association.

Selectivity issues. As for any of the compounds currently used in chemotherapy, inhibitors of CDC25 phosphatases are not envisaged to be selective for tumour cells, and therefore would probably inhibit the cell-cycle progression of any cell type. However, the upregulation of CDC25A and CDC25B found in various types of tumours (as discussed above) suggest that increased sensitivity could exist in these cases. The use of cells that have been genetically engineered to overexpress CDC25B has allowed us to validate this hypothesis. HCT116 colon adenocarcinoma cell lines that express an increased level of CDC25B are more sensitive to the inhibition of CDC25 activity by chemical compounds (B. Aressy, B. Bugler and B.D., unpublished observations). Pancreatic ductal adenocarcinoma cell lines that express high levels of CDC25B were also found to be more sensitive to growth inhibition by CDC25 inhibitors[102]. These observations should now be validated in studies with primary tumour samples.

The additional questions, which are a source of debate in the field, are first, whether or not it is necessary to selectively target CDC25 isoforms with specific inhibitors and, second, whether it is possible to identify such specific compounds. With respect to the first question, a pragmatic answer is that the potent antiproliferative activity observed with the global inhibition of all three CDC25 isoforms, is in favour of a multitargeted compound approach. A second answer with reference to the abnormal CDC25 expression pattern (see above) observed in tumours would be to consider the possibility of selectively inhibiting either CDC25A or CDC25B. Finally, a cautious answer would be to say that the global inhibition of CDC25 is a way to circumvent the possible functional redundancy that is suggested by knockdown experiments in animal models. With regard to the second

question, it is quite easy to answer that in the current state of knowledge (or lack of knowledge) of the CDC25 enzymes and in particular, in the absence of information on the three-dimensional structure of the regulatory domain (which is the most divergent part of the three isoforms), it is very difficult to envisage the rational design of isoform-specific compounds. The very few published inhibitors that claim to be specific for one of the three CDC25s[154] show IC_{50} values that are probably in the range of the experimental variability inherent in the difficult task of obtaining pure, homogenous and comparable CDC25A, B and C enzyme preparations.

Is CDC25 activation a feasible approach? Is it worth increasing the activities of CDC25 proteins rather than decreasing them? Why should such an approach be more efficient in killing cancer cells? As discussed above, the expression level of CDC25B seems to be a key factor in the ability of the cells to recover from the G2/M checkpoint, activated by genotoxic damage. In fact, experimentally, very moderate increases in the level of CDC25B associated with a DNA-damaging treatment significantly increases the sensitivity of tumour cells to doxorubicin or ionizing radiation (B. Aressy, B. Bugler and B.D., unpublished observations). In a situation where the G2/M checkpoint has been switched on by DNA damage response pathways, the activation of mitosis-inducing activity would push cells that have not yet repaired their genome into unscheduled and catastrophic mitoses. Thus, inhibiting the DNA damage checkpoint response pathway through the activation of CDC25 phosphatases might be of major interest as an adjuvant to radiotherapy or to treatment with DNA-damaging drugs. The idea would be to radiosensitize or chemosensitize tumours by forcing cancer cells arrested at the DNA-damage-activated checkpoint to continue through the cell cycle and thus commit suicide.

Compounds that act directly to increase the catalytic activities of CDC25 phosphatases have not yet been reported. The pharmacological application of the checkpoint bypass concept relies on the use of compounds that inhibit the activities of the upstream kinases that negatively control CDC25 activity, that is to say, the checkpoint kinase family. Inhibitors such as UCN-01, which is poorly selective[75,155], and potentially more selective compounds[156,157] such as XL-844, a compound that has recently entered phase I clinical trials[158], are currently being studied. Inhibition of the p38–MAPKAPK2 pathway might also be of great interest, as suggested by the recent demonstration of its involvement in the regulation of the CDC25s in G2/M checkpoint arrest in cancer cells treated with DNA-damaging anticancer agents[75]. In reversing the inhibition of CDC25 phosphatases, these compounds might provide an appealing strategy in association with genotoxic or ionizing radiation. The relevance of this approach is further reinforced by the increasing experimental evidence for G2/M checkpoint reinforcement in cancer stem cells, with radioresistance and chemoresistance associated with increased CHK1 activity. This has recently been shown in glioma $CD133^+$ stem cells, which are efficiently radiosensitized by the use of a checkpoint-abrogating compound[159].

However, the question of the potential effects of such compounds on genome stability, both in targeted cancer cells and in normal tissues, has to be carefully considered. It is established that checkpoint kinases also have a constitutive role in negatively regulating the activity of CDC25 in the absence of a DNA insult, in order to control progression through the cell cycle and preserve genomic integrity[50,51]. One can imagine that the inhibition of CHK activity might therefore be deleterious, and favour mutagenesis and carcinogenic transformation. Furthermore, when associated with a genotoxic treatment or ionizing radiation, CHK inhibitors will favour checkpoint bypass. If the outcome of this bypass does not result in cancer cell death, such a strategy might lead to the acquisition of genomic aberrations and potentially more aggressive characteristics.

At this stage, it is obviously difficult to make predictions of the comparative values of these two pharmacological approaches. Bearing in mind the fact that what matters most is the efficacy of the therapeutic approach on the tumour, and the safety to the patient, both strategies will have to be further validated in experimental model systems and large-scale studies.

Future directions

A critical assessment of the existing data on CDC25 phosphatases in cancer raises several issues, which should ideally be addressed in future studies. A standardized approach to measuring CDC25 expression, with a clear definition of the normal tissue-specific threshold levels, would certainly facilitate comparative analyses between studies and improve the value of subsequent correlations with clinicopathological features and modes of treatment. In addition, the consideration of splice variant-specific changes in CDC25 levels and their involvement in cancer have been neglected in previous studies, largely owing to technical limitations. Surmounting such difficulties, and combining this information with ongoing analyses in cell line models, will be essential for a complete understanding of the relative roles of not only each CDC25 isoform, but also of specific splice variants in both unperturbed and perturbed cell division. The dissolution of these issues will dictate whether CDC25 expression can be used as a reliable prognostic indicator in the clinic, to be taken into account when choosing the most effective therapeutic strategy for the patient at hand. Furthermore, compounds that inhibit CDC25 phosphatases and show potent inhibitory activity towards cancer cells will continue to be evaluated, particularly with respect to their applicability in combination therapy approaches. As presented, one can also envisage that alternative means of targeting CDC25 phosphatases, such as the activation of their catalytic activities, as is currently under investigation for other checkpoint targets, will be assessed for their potential as anticancer agents. Recent advances in the CDC25 field have led to the identification of promising pharmacological tools for cancer treatment, but it is difficult to predict which strategy will be more successful. The best therapeutic indications for CDC25 inhibition and checkpoint abrogation are still open questions that will require validation by a better understanding of CDC25 biology, as well as at the clinical trial level.

Radioresistance
A property of cells that are intrinsically resistant to usual doses of ionizing radiation.

1. Boudolf, V., Inze, D. & De Veylder, L. What if higher plants lack a CDC25 phosphatase? *Trends Plant Sci.* **11**, 474–479 (2006).
2. Galaktionov, K. & Beach, D. Specific activation of cdc25 tyrosine phosphatases by B-type cyclins: evidence for multiple roles of mitotic cyclins. *Cell* **67**, 1181–1194 (1991).
3. Sadhu, K., Reed, S. I., Richardson, H. & Russell, P. Human homolog of fission yeast cdc25 mitotic inducer is predominantly expressed in G2. *Proc. Natl Acad. Sci. USA* **87**, 5139–5143 (1990).
4. Nagata, A., Igarashi, M., Jinno, S., Suto, K. & Okayama, H. An additional homolog of the fission yeast *cdc25* gene occurs in humans and is highly expressed in some cancer cells. *The new Biologist* **3**, 959–968 (1991).
This was the first study reporting the existence of multiple human homologues of the yeast *cdc25*$^{+}$ gene and also the first report of CDC25 overexpression in cancer cell lines.
5. Izumi, T. & Maller, J. L. Elimination of cdc2 phosphorylation sites in the cdc25 phosphatase blocks initiation of M-phase. *Mol. Cell Biol.* **4**, 1337–1350 (1993).
6. Okazaki, K. *et al.* Isolation of a cDNA encoding the *xenopus* homologue of mammalian Cdc25A that can induce meiotic maturation of oocytes. *Gene* **178**, 111–114 (1996).
7. Benazeraf, B. *et al.* Identification of an unexpected link between the Shh pathway and a G2/M regulator, the phosphatase CDC25B. *Dev. Biol.* **294**, 133–147 (2006).
8. Wegener, S., Hampe, W., Herrmann, D. & Schaller, H. C. Alternative splicing in the regulatory region of the human phosphatases CDC25A and CDC25C. *Eur. J. Cell Biol.* **79**, 810–815. (2000).
9. Baldin, V., Cans, C., Superti-Furga, G. & Ducommun, B. Alternative splicing of the human CDC25B tyrosine phoshatase. Possible implications for growth control? *Oncogene* **14**, 2485–2495 (1997).
The first report that multiple CDC25B variants derive from a single mRNA by alternative splicing events.
10. Forrest, A. R. *et al.* Multiple splicing variants of cdc25B regulate G2/M progression. *Biochem. Biophys. Res. Commun.* **260**, 510–515 (1999).
11. Bureik, M. *et al.* An additional transcript of the cdc25C gene from A431 cells encodes a functional protein. *Int. J. Oncol.* **17**, 1251–1258. (2000).
12. Russell, P. & Nurse, P. cdc25 + functions as an inducer in the mitotic control of fission yeast. *Cell* **45**, 145–153 (1986).
13. Malumbres, M. & Barbacid, M. Mammalian cyclin-dependent kinases. *Trends Biochem. Sci.* **30**, 630–641 (2005).
14. Kaldis, P. The cdk-activating kinase (CAK): from yeast to mammals. *Cell Mol. Life Sci.* **55**, 284–296 (1999).
15. Kristjansdottir, K. & Rudolph, J. Cdc25 phosphatases and cancer. *Chem. Biol.* **11**, 1043–1051 (2004).
A comprehensive review on the different methods used to detect CDC25 overexpression in human cancer.
16. Blomberg, I. & Hoffmann, I. 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 (1999).
17. Hoffman, I., Draetta, I. & Karsenti, G. 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 (1994).
18. Jinno, S. *et al.* Cdc25A is a novel phosphatase functioning early in the cell cycle. *EMBO J.* **13**, 1549–1556 (1994).
19. Molinari, M., Mercurio, C., Dominguez, J., Goubin, F. & Draetta, G. F. Human Cdc25 A inactivation in response to S phase inhibition and its role in preventing premature mitosis. *EMBO Rep.* **1**, 71–79. (2000).
20. Zhao, H., Watkins, J. L. & Piwnica-Worms, H. Disruption of the checkpoint kinase 1/cell division cycle 25A pathway abrogates ionizing radiation-induced S and G2 checkpoints. *Proc. Natl Acad. Sci. USA* **99**, 14795–14800 (2002).
21. Lindqvist, A., Kallstrom, H., Lundgren, A., Barsoum, E. & Rosenthal, C. K. 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 (2005).
An important study exploring the relative roles of CDC25A and CDC25B in inducing mitotic onset. The authors show that CDC25B is required for the initial activation of CDK1–cyclin B at the centrosomes, whereas CDC25A is involved in chromatin condensation.
22. Mailand, N. *et al.* Regulation of G(2)/M events by Cdc25A through phosphorylation-dependent modulation of its stability. *EMBO J.* **21**, 5911–5920 (2002).
23. Boutros, R., Dozier, C. & Ducommun, B. The when and wheres of CDC25 phosphatases. *Curr. Opin. Cell Biol.* **18**, 185–191 (2006).
A detailed review of the complex mechanisms that regulate CDC25 activities throughout the cell cycle.
24. Millar, J. *et al.* p55 cdc25 is a nuclear phosphoprotein required for the initiation of mitosis in human cells. *Proc. Natl Acad. Sci. USA* **88**, 10500–10504 (1991).
25. Lammer, C. *et al.* The cdc25B phosphatase is essential for the G2/M phase transition in human cells. *J. Cell Sci.* **111**, 2445–2453 (1998).
26. Gabrielli, B. G. *et al.* Cytoplasmic accumulation of CDC25B phosphatase in mitosis triggers centrosomal microtubule nucleation in HeLa cells. *J. Cell Sci.* **109**, 1081–1093 (1996).
The first demonstration of a link between cytoplasmic CDC25B accumulation and centrosomal events that occur during the G2–M transition.
27. De Souza, C. P., Ellem, K. A. & Gabrielli, B. G. Centrosomal and cytoplasmic Cdc2/cyclin B1 activation precedes nuclear mitotic events. *Exp. Cell Res.* **257**, 11–21 (2000).
28. Gabrielli, B. G., Clarck, J. M., McCormack, A. & Ellem, K. A. O. Hyperphosphorylation of the N-terminal domain of cdc25 regulates activity toward cyclinB1/cdc2 but not cyclin A/cdk2. *J. Biol. Chem.* **272**, 28607–28614 (1997).
29. Turowski, P. *et al.* Functional cdc25C dual-specificity phosphatase is required for S-phase entry in human cells. *Mol. Biol. Cell* **14**, 2984–2998 (2003).
30. Garner-Hamrick, P. A. & Fischer, C. Antisense phosphoorthioate oligonucleotides specifically down-regulate cdc25B causing S-phase delay and persitent antiproliferative effects. *Int. J. Cancer* **76**, 720–728 (1998).
31. Ferguson, A. M., White, L. S., Donovan, P. J. & Piwnica-Worms, H. Normal cell cycle and checkpoint responses in mice and cells lacking Cdc25B and Cdc25C protein phosphatases. *Mol. Cell Biol.* **25**, 2853–2860 (2005).
An important study in which the authors challenge the idea that all three CDC25 isoforms are required for normal cell-cycle progression, by successfully generating mice that lack both CDC25B and CDC25C without any obvious defects in cell-cycle function or checkpoint responses.
32. Lincoln, A. J. *et al.* Cdc25b phosphatase is required for resumption of meiosis during oocyte maturation. *Nature Genet.* **30**, 446–449 (2002).
An interesting study showing that *Cdc25b*$^{-/-}$ female mice are sterile because of permanent meiotic arrest, resulting from lack of maturation promoting factor (MPF; CDK1-cyclinB1 complex) activity.
33. Karlsson-Rosenthal, C. & Millar, J. B. Cdc25: mechanisms of checkpoint inhibition and recovery. *Trends Cell Biol.* **16**, 285–292 (2006).
34. Cazales, M. *et al.* CDC25B phosphorylation by Aurora-A occurs at the G2/M transition and is inhibited by DNA damage. *Cell Cycle* **4**, 1233–1238 (2005).
35. Dutertre, S. *et al.* Phosphorylation of CDC25B by Aurora-A at the centrosome contributes to the G2/M transition. *J. Cell Sci.* **117**, 2523–2531 (2004).
This study demonstrates that phosphorylation by Aurora A is required for CDC25B activity and localization to the centrosomes to initiate mitotic entry.
36. Theis-Febvre, N. *et al.* Protein kinase CK2 regulates CDC25B phosphatase activity. *Oncogene* **22**, 220–232 (2003).
37. Hoffmann, I., Clarke, P., Marcote, M. J., Karsenti, E. & Draetta, G. Phosphorylation and activation of human cdc25-C by cdc2-cyclin B and its involvement in the self amplification of MPF at mitosis. *EMBO J.* **12**, 53–63 (1993).
38. Barr, F. A., Sillje, H. H. & Nigg, E. A. Polo-like kinases and the orchestration of cell division. *Nature Rev. Mol. Cell Biol.* **5**, 429–440 (2004).
39. Karaiskou, A., Jessus, C., Brassac, T. & Ozon, R. Phosphatase 2A and polo kinase, two antagonistic regulators of cdc25 activation and MPF auto-amplification. *J Cell Sci.* **112**, 3747–3756 (1999).
40. Kumagaï, A. & Dunphy, W. G. Purification and molecular cloning of Plx1, a Cdc25-reg ulatory kinase from Xenopus egg extracts. *Science* **273**, 1377–1380 (1996).
41. Qian, Y. W., Erikson, E., Li, C. & Maller, J. L. Activated polo-like kinase Plx1 is required at multiple points during mitosis in *Xenopus laevis*. *Mol. Cell Biol.* **18**, 4262–4271 (1998).
42. Roshak, A. K. *et al.* The human polo-like kinase, PLK, regulates cdc2/cyclin B through phosphorylation and activation of the cdc25C phosphatase. *Cell Signal.* **12**, 405–411 (2000).
43. Myer, D. L., Bahassi el, M. & Stambrook, P. J. The Plk3-Cdc25 circuit. *Oncogene* **24**, 299–305 (2005).
44. Toyoshima-Morimoto, F., Taniguchi, E. & Nishida, E. Plk1 promotes nuclear translocation of human Cdc25C during prophase. *EMBO Rep.* **3**, 341–348 (2002).
45. Hermeking, H. & Benzinger, A. 14–3-3 proteins in cell cycle regulation. *Semin. Cancer Biol.* **16**, 183–192 (2006).
46. Davezac, N. *et al.* Regulation of CDC25B phosphatases subcellular localization. *Oncogene* **19**, 2179–2185 (2000).
47. Loffler, H. *et al.* Chk1-dependent regulation of Cdc25B functions to coordinate mitotic events. *Cell Cycle* 1 November 2006 (Epub ahead of print).
48. Mils, V. *et al.* Specific interaction between 14. 3. 3 isoforms and the human CDC25B phosphatase. *Oncogene* **19**, 1257–1265 (2000).
49. Sanchez, Y. *et al.* Conservation of the Chk1 checkpoint pathway in mammals: linkage of DNA damage to Cdk regulation through Cdc25. *Science* **277**, 1497–1501 (1997).
50. Kramer, A. *et al.* Centrosome-associated Chk1 prevents premature activation of cyclin-B-Cdk1 kinase. *Nature Cell Biol.* **6**, 884–891 (2004).
An interesting study in which CHK1 is reported to localize to the centrosomes of interphase cells, and this pool of CHK1 functions to prevent the premature activation of CDK1–cyclin B.
51. Schmitt, E. *et al.* CHK1 phosphorylates CDC25B during the cell cycle in the absence of DNA damage. *J. Cell Sci.* **119**, 4269–4275 (2006).
This study shows that CHK1 phosphorylates CDC25B *in vitro* and *in vivo* during interphase in the absence of DNA damage, in order to inhibit its mitosis-inducing activity.
52. Mirey, G. *et al.* CDC25B phosphorylated by pEg3 localizes to the centrosome and the spindle poles at mitosis. *Cell Cycle* **4**, 806–811 (2005).
53. Chen, M. S., Ryan, C. E. & Piwnica-Worms, H. Chk1 kinase negatively regulates mitotic function of Cdc25A phosphatase through 14–3-3 binding. *Mol. Cell Biol.* **23**, 7488–7497 (2003).
54. Donzelli, M. *et al.* Dual mode of degradation of Cdc25 A phosphatase. *EMBO J.* **21**, 4875–4884 (2002).
55. Busino, L., Chiesa, M., Draetta, G. F. & Donzelli, M. Cdc25A phosphatase: combinatorial phosphorylation, ubiquitylation and proteolysis. *Oncogene* **23**, 2050–2056 (2004).
56. Busino, L. *et al.* Degradation of Cdc25A by β-TrCP during S phase and in response to DNA damage. *Nature* **426**, 87–91 (2003).
57. Jin, J. *et al.* SCFβ-TRCP links Chk1 signaling to degradation of the Cdc25A protein phosphatase. *Genes Dev.* **17**, 3062–3074 (2003).
58. Donzelli, M. *et al.* Hierarchical order of phosphorylation events commits Cdc25A to βTrCP-dependent degradation. *Cell Cycle* **3**, 469–471 (2004).
59. Baldin, V., Cans, C., Knibiehler, M. & Ducommun, B. Phosphorylation of human CDC25B phosphatase by CDK1/cyclin A triggers its proteasome-dependent degradation. *J. Biol. Chem.* **272**, 32731–32735 (1997).
60. Kanemori, Y., Uto, K. & Sagata, N. β-TrCP recognizes a previously undescribed nonphosphorylated destruction motif in Cdc25A and Cdc25B phosphatases. *Proc. Natl Acad. Sci. USA* **102**, 6279–6284 (2005).
61. Hurley, P. J. & Bunz, F. ATM and ATR: components of an integrated circuit. *Cell Cycle* **6**, 414–417 (2007).
62. Niida, H. & Nakanishi, M. DNA damage checkpoints in mammals. *Mutagenesis* **21**, 3–9 (2006).
63. Goloudina, A. *et al.* Regulation of human Cdc25A stability by Serine 75 phosphorylation is not sufficient to activate a S phase checkpoint. *Cell Cycle* **2**, 473–478 (2003).

64. Xiao, Z. *et al.* Chk1 mediates S and G2 arrests through Cdc25A degradation in response to DNA-damaging agents. *J. Biol. Chem.* **278**, 21767–21773 (2003).
65. Mailand, N. *et al.* Rapid destruction of human Cdc25A in response to DNA damage. *Science* **288**, 1425–1429 (2000).
66. Sorensen, C. S. *et al.* Chk1 regulates the S phase checkpoint by coupling the physiological turnover and ionizing radiation-induced accelerated proteolysis of Cdc25A. *Cancer Cell* **3**, 247–258 (2003).
67. Matsuoka, S., Huang, M. & Elledge, S. J. Linkage of ATM to cell cycle regulation by the Chk2 protein kinase. *Science* **282**, 1893–1897 (1998).
68. Peng, C. Y. *et al.* Mitotic and G2 checkpoint control: regulation of 14. 3. 3 protein binding by phosphorylation of CDC25C on Serine-216. *Science* **277**, 1501–1505 (1997).
69. Yarden, R. I., Pardo-Reoyo, S., Sgagias, M., Cowan, K. H. & Brody, L. C. BRCA1 regulates the G2/M checkpoint by activating Chk1 kinase upon DNA damage. *Nature Genet.* **30**, 285–289 (2002).
70. Raleigh, J. M. & O'Connell, M. J. The G(2) DNA damage checkpoint targets both Wee1 and Cdc25. *J. Cell Sci.* **113**, 1727–1736 (2000).
71. Mikhailov, A., Shinohara, M. & Rieder, C. L. The p38-mediated stress-activated checkpoint. A rapid response system for delaying progression through antephase and entry into mitosis. *Cell Cycle* **4**, 57–62 (2005).
72. Bulavin, D. V. *et al.* Initiation of a G2/M checkpoint after ultraviolet radiation requires p38 kinase. *Nature* **411**, 102–107 (2001).
The first study to show the essential role of the p38 kinase in the G2/M checkpoint response to UV radiation, by the phosphorylation of both CDC25B and CDC25C, and by the inhibition of 14-3-3 binding in the case of CDC25B.
73. Lemaire, M. *et al.* CDC25B Phosphorylation by p38 and MK-2. *Cell Cycle* **5**, 1649–1653 (2006).
74. Manke, I. A. *et al.* MAPKAP kinase-2 is a cell cycle checkpoint kinase that regulates the G2/M transition and S phase progression in response to UV irradiation. *Mol. Cell* **17**, 37–48 (2005).
75. Reinhardt, H. C., Aslanian, A. S., Lees, J. A. & Yaffe, M. B. p53-deficient cells rely on ATM- and ATR-mediated checkpoint signaling through the p38MAPK/MK2pathway for survival after DNA damage. *Cancer Cell* **11**, 175–189 (2007).
A very interesting study in which the p38–MAPKAPK2 pathway was shown to function as an alternative checkpoint response to UV in p53-deficient tumour cells, and to be directly activated by DNA-damaging chemotherapeutic agents.
76. van Vugt, M. A., Bras, A. & Medema, R. H. Polo-like kinase-1 controls recovery from a G2 DNA damage-induced arrest in mammalian cells. *Mol. Cell* **15**, 799–811 (2004).
In this study, the authors show that although PLK1 is not an essential kinase for mitotic entry in undamaged cells, it becomes indispensable along with CDC25B, for the recovery of G2/M checkpoint arrest after DNA damage.
77. Bugler, B. *et al.* Genotoxic-activated G2-M checkpoint exit is dependent on CDC25B phosphatase expression. *Mol. Cancer Ther.* **5**, 1446–1451 (2006).
78. Bansal, P. & Lazo, J. S. Induction of Cdc25B regulates cell cycle resumption after genotoxic stress. *Cancer Res.* **67**, 3356–3363 (2007).
79. Cangi, M. G. *et al.* Role of the Cdc25A phosphatase in human breast cancer. *J. Clin. Invest.* **106**, 753–761 (2000).
80. Bonin, S., Brunetti, D., Benedetti, E., Gorji, N. & Stanta, G. Expression of cyclin-dependent kinases and CDC25a phosphatase is related with recurrences and survival in women with peri- and post-menopausal breast cancer. *Virchows Arch.* **448**, 539–544 (2006).
81. Ito, Y. *et al.* Expression of cdc25A and cdc25B phosphatase in breast carcinoma. *Breast Cancer* **11**, 295–300 (2004).
82. Galaktionov, K. *et al.* Cdc25 phosphatases as potential human oncogenes. *Science* **269**, 1575–1577 (1995).
This study was the first report of overexpression of CDC25B in primary tissue samples from human breast cancer, and also the first to associate this overexpression with a higher tumour grade.
83. Broggini, M. *et al.* Cell cycle-related phosphatases CDC25A and B expression correlates with survival in ovarian cancer patients. *Anticancer Res.* **20**, 4835–4840 (2000).
84. Ngan, E. S., Hashimoto, Y., Ma, Z. Q., Tsai, M. J. & Tsai, S. Y. Overexpression of Cdc25B, an androgen receptor coactivator, in prostate cancer. *Oncogene* **22**, 734–739 (2003).
85. Ozen, M. & Ittmann, M. Increased expression and activity of CDC25C phosphatase and an alternatively spliced variant in prostate cancer. *Clin. Cancer Res.* **11**, 4701–4706 (2005).
86. Sasaki, H. *et al.* Expression of the *cdc25B* gene as a prognosis marker in non-small cell lung cancer. *Cancer Lett.* **173**, 187–192 (2001).
87. Wu, W., Fan, Y. H., Kemp, B. L., Walsh, G. & Mao, L. Overexpression of cdc25A and cdc25B is frequent in primary non-small cell lung cancer but is not associated with overexpression of c-myc. *Cancer Res.* **58**, 4082–4085 (1998).
88. Takemasa, I. *et al.* Overexpression of CDC25B phosphatase as a novel marker of poor prognosis of human colorectal carcinoma. *Cancer Res.* **60**, 3043–3050 (2000).
89. Hernandez, S. *et al.* Differential expression of cdc25 cell-cycle-activating phosphatases in human colorectal carcinoma. *Lab. Invest.* **81**, 465–473 (2001).
90. Talvinen, K. *et al.* Biochemical and clinical approaches in evaluating the prognosis of colon cancer. *Anticancer Res.* **26**, 4745–4751 (2006).
91. Hu, Y. C., Lam, K. Y., Law, S., Wong, J. & Srivastava, G. Identification of differentially expressed genes in esophageal squamous cell carcinoma (ESCC) by cDNA expression array: overexpression of *Fra-1*, Neogenin, *Id-1*, and *CDC25B* genes in ESCC. *Clin. Cancer Res.* **7**, 2213–2221 (2001).
92. Miyata, H. *et al.* CDC25B and p53 are independently implicated in radiation sensitivity for human esophageal cancers. *Clin. Cancer Res.* **6**, 4859–4865 (2000).
93. Nishioka, K. *et al.* Clinical significance of CDC25A and CDC25B expression in squamous cell carcinomas of the oesophagus. *Br. J. Cancer* **85**, 412–421 (2001).
94. Kishi, K. *et al.* Prediction of the response to chemoradiation and prognosis in oesophageal squamous cancer. *Br. J. Surg.* **89**, 597–603 (2002).
95. Sunada, F., Itabashi, M., Ohkura, H. & Okumura, T. p53 negativity, CDC25B positivity, and metallothionein negativity are predictors of a response of esophageal squamous cell carcinoma to chemoradiotherapy. *World J. Gastroenterol.* **11**, 5696–5700 (2005).
96. Ito, Y. *et al.* Cdc25A and cdc25B expression in malignant lymphoma of the thyroid: correlation with histological subtypes and cell proliferation. *Int. J. Mol. Med.* **13**, 431–435 (2004).
97. Ito, Y. *et al.* Expression of cdc25A and cdc25B proteins in thyroid neoplasms. *Br. J. Cancer* **86**, 1909–1913 (2002).
98. Ito, Y. *et al.* Expression of cdc25B and cdc25A in medullary thyroid carcinoma: cdc25B expression level predicts a poor prognosis. *Cancer Lett.* **229**, 291–297 (2005).
99. Fraczek, M., Wozniak, Z., Ramsey, D. & Krecicki, T. Expression patterns of cyclin E, cyclin A and CDC25 phosphatases in laryngeal carcinogenesis. *Eur. Arch. Otorhinolaryngol.* 2007 (doi:10-1007/s00405-007-0276-2)
100. Xu, X. *et al.* Overexpression of CDC25A phosphatase is associated with hypergrowth activity and poor prognosis of human hepatocellular carcinomas. *Clin. Cancer Res.* **9**, 1764–1772 (2003).
101. Kudo, Y. *et al.* Overexpression of cyclin-dependent kinase activating CDC25B phosphatase in human gastric carcinomas. *Jpn J. Cancer Res.* **88**, 947–952 (1997).
102. Guo, J. *et al.* Expression and functional significance of CDC25B in human pancreatic ductal adenocarcinoma. *Oncogene* **23**, 71–81 (2004).
103. Wu, W. *et al.* Coordinate expression of Cdc25B and ER-α is frequent in low-grade endometrioid endometrial carcinoma but uncommon in high-grade endometrioid and nonendometrioid carcinomas. *Cancer Res.* **63**, 6195–6199 (2003).
104. Tsuda, H., Hashiguchi, Y., Inoue, T. & Yamamoto, K. Alteration of G2 cell cycle regulators occurs during carcinogenesis of the endometrium. *Oncology* **65**, 159–166 (2003).
105. Gasparotto, D. *et al.* Overexpression of CDC25A and CDC25B in head and neck cancers. *Cancer Res.* **57**, 2366–2368 (1997).
106. Sato, Y. *et al.* Expression of the cdc25B mRNA correlated with that of N-myc in neuroblastoma. *Jpn J. Clin. Oncol.* **31**, 428–431 (2001).
107. Nakabayashi, H., Hara, M. & Shimizu, K. Prognostic significance of CDC25B expression in gliomas. *J. Clin. Pathol.* **59**, 725–728 (2006).
108. Hernandez, S. *et al.* CDC25 cell cycle-activating phosphatases and c-myc expresion in human non-hodgkin's lymphomas. *Cancer Res.* **58**, 1762–1767 (1998).
109. Hernandez, S. *et al.* cdc25a and the splicing variant cdc25b2, but not cdc25B1, -B3 or-C, are over-expressed in aggressive human non-Hodgkin's lymphomas. *Int. J. Cancer* **89**, 148–152 (2000).
110. Moreira, G. Jr. *et al.* Reciprocal CDC25A and p27 expression in B-cell non Hodgkin lymphomas. *Diagn. Mol. Pathol.* **12**, 128–132 (2003).
111. Aref, S. *et al.* c-Myc oncogene and Cdc25A cell activating phosphatase expression in non-Hodgkin's lymphoma. *Hematology* **8**, 183–190 (2003).
112. Nishioka, K. *et al.* Clinical significance of CDC25A and CDC25B expression in squamous cell carcinomas of the oesophagus. *Br. J. Cancer* **85**, 412–421 (2001).
113. Galaktionov, K., Chen, X. C. & Beach, D. Cdc25 cell-cycle phosphatase as a target of c-myc. *Nature* **382**, 511–517 (1996).
114. Ben-Yosef, T., Yanuka, O., Halle, D. & Benvenisty, N. Involvement of Myc targets in c-myc and N-myc induced human tumors. *Oncogene* **17**, 165–171 (1998).
115. Vigo, E. *et al.* CDC25A phosphatase is a target of E2F and is required for efficient E2F-induced S phase. *Mol. Cell Biol.* **19**, 6379–6395 (1999).
116. Wu, L. *et al.* E2F-Rb complexes assemble and inhibit cdc25A transcription in cervical carcinoma cells following repression of human papillomavirus oncogene expression. *Mol. Cell Biol.* **20**, 7059–7067 (2000).
117. Spitkovsky, D., Jansen-Dürr, P., Karsenti, E. & Hoffmann, I. S-phase induction by adenovirus E1A requires activation of cdc25A tyrosine phosphatase. *Oncogene* **12**, 2549–2554 (1996).
118. Loffler, H. *et al.* Distinct modes of deregulation of the proto-oncogenic Cdc25A phosphatase in human breast cancer cell lines. *Oncogene* **22**, 8063–8071 (2003).
119. Alderton, G. K. *et al.* Regulation of mitotic entry by microcephalin and its overlap with ATR signalling. *Nature Cell Biol.* **8**, 725–733 (2006).
120. Gerstein, A. V. *et al.* APC/CTNNB1 (β-catenin) pathway alterations in human prostate cancers. *Genes Chromosomes Cancer* **34**, 9–16 (2002).
121. He, N. *et al.* Regulation of lung cancer cell growth and invasiveness by β-TRCP. *Mol. Carcinog.* **42**, 18–28 (2005).
122. Kim, C. J. *et al.* Somatic mutations of the β-TrCP gene in gastric cancer. *Apmis* **115**, 127–133 (2007).
123. Bartek, J. & Lukas, J. Chk1 and Chk2 kinases in checkpoint control and cancer. *Cancer Cell* **3**, 421–429 (2003).
124. Kim, S. O. *et al.* Differential expression of Csk homologous kinase (CHK) in normal brain and brain tumors. *Cancer* **101**, 1018–1027 (2004).
125. Tort, F. *et al.* Checkpoint kinase 1 (CHK1) protein and mRNA expression is downregulated in aggressive variants of human lymphoid neoplasms. *Leukemia* **19**, 112–117 (2005).
126. Ray, D. *et al.* Deregulated CDC25A expression promotes mammary tumorigenesis with genomic instability. *Cancer Res.* **67**, 984–991 (2007).
In this study, the authors show that although transgenic expression of CDC25A alone is not sufficient to induce spontaneous tumour growth in mice, CDC25A overexpression generates genomic instability and cooperates with v-Ha-*ras* or c-*neu* oncogenes in murine mammary tumorigenesis.
127. Ma, Z. Q., Chua, S. S., DeMayo, F. J. & Tsai, S. Y. Induction of mammary gland hyperplasia in transgenic mice over- expressing human cdc25B. *Oncogene* **18**, 4564–4576 (1999).
128. Yao, Y. *et al.* Increased susceptibility to carcinogen-induced mammary tumors in MMTV- Cdc25B transgenic mice. *Oncogene* **18**, 5159–5166 (1999).
References 127 and 128 discuss the effect of CDC25B overexpression in inducing mammary tumour growth. The expression of CDC25B alone promotes proliferation and hyperplasia but requires the presence of a carcinogen to stimulate tumour growth.
129. Karlsson, C., Katich, S., Hagting, A., Hoffmann, I. & Pines, J. Cdc25B and Cdc25C differ markedly in their properties as initiators of mitosis. *J. Cell Biol.* **146**, 573–584 (1999).
130. Sexl, V. *et al.* A rate limiting function of cdc25A for S phase entry inversely correlates with tyrosine dephosphorylation of Cdk2. *Oncogene* **18**, 573–582 (1999).

131. Falck, J., Mailand, N., Syljuasen, R. G., Bartek, J. & Lukas, J. The ATM-Chk2-CDC25A checkpoint pathway guards against radioresistant DNA synthesis. *Nature* **410**, 842–847. (2001).
132. Senderowicz, A. M. Targeting cell cycle and apoptosis for the treatment of human malignancies. *Curr. Opin. Cell Biol.* **16**, 670–678 (2004).
133. Senderowicz, A. M. Inhibitors of cyclin-dependent kinase modulators for cancer therapy. *Prog. Drug Res.* **63**, 183–206 (2005).
134. Byrd, J. C. *et al.* Flavopiridol administered using a pharmacologically derived schedule is associated with marked clinical efficacy in refractory, genetically high-risk chronic lymphocytic leukemia. *Blood* **109**, 399–404 (2007).
135. Benson, C. *et al.* A phase I trial of the selective oral cyclin-dependent kinase inhibitor seliciclib (CYC202; R-Roscovitine), administered twice daily for 7 days every 21 days. *Br. J. Cancer* **96**, 29–37 (2007).
136. Prevost, G. P. *et al.* in *Progress in Cell Cycle Research* (eds Meijer, L., Jézéquel, A. & Roberge, M.) 225–234 (Life in progress, Roscoff, France, 2003).
137. Lazo, J. S. *et al.* Discovery and biological evaluation of a new family of potent inhibitors of the dual specificity protein phosphatase Cdc25. *J. Med. Chem.* **44**, 4042–4049 (2001).
This study shows how the screening of a compound library coupled with experimental and computational approaches can result in the identification of a potent and specific CDC25 inhibitor.
138. Brisson, M. *et al.* Discovery and characterization of novel small molecule inhibitors of human Cdc25B dual specificity phosphatase. *Mol. Pharmacol.* **66**, 824–833 (2004).
139. Cao, S., Foster, C., Brisson, M., Lazo, J. S. & Kingston, D. G. Halenaquinone and xestoquinone derivatives, inhibitors of Cdc25B phosphatase from a *Xestospongia* sp. *Bioorg. Med. Chem.* **13**, 999–1003 (2005).
140. Brezak, M. C. *et al.* Inhibition of human tumor cell growth *in vivo* by an orally bioavailable inhibitor of CDC25 phosphatases. *Mol. Cancer Ther.* **4**, 1378–1387 (2005).
An important study reporting the identification of BN82685, a quinone-based potent CDC25 inhibitor. The fact that this compound inhibits the growth of xenografted human pancreatic tumours when orally administered highlights the value of CDC25 phosphatases as targets for anticancer therapeutics.
141. 141. Galcera, M. O., Bigg, D., Prevost, G. & Sidhu, A. CDC25 phosphatases inhibitors. WO 2006/067311 (2006).
142. Kar, S., Wang, M., Ham, S. W. & Carr, B. I. H32, a non-quinone sulfone analog of vitamin K3, inhibits human hepatoma cell growth by inhibiting Cdc25 and activating ERK. *Cancer Biol. Ther.* **5**, 1340–1347 (2006).
143. Blanchard, J. L., Epstein, D. M., Boisclair, M. D., Rudolph, J. & Pal, K. Dysidiolide and related γ-hydroxy butenolide compounds as inhibitors of the protein tyrosine phosphatase, CDC25. *Bioorg. Med. Chem. Lett.* **9**, 2537–2538 (1999).
144. Ham, S. W. *et al.* Naphthoquinone analogs as inactivators of cdc25 phosphatase. *Bioorg. Med. Chem. Lett.* **8**, 2507–2510 (1998).
145. Wrobel, J. *et al.* PTP1B inhibition and antihyperglycemic activity in the ob/ob mouse model of novel 11-arylbenzo[b]naphtho[2, 3-d]furans and 11-arylbenzo[b]naphtho[2, 3-d]thiophenes. *J. Med. Chem.* **42**, 3199–3202 (1999).
146. Tamura, K. *et al.* Cdc25 inhibition and cell cycle arrest by a synthetic thioalkyl vitamin K analogue. *Cancer Res.* **60**, 1317–1325 (2000).
147. Sohn, J. & Rudolph, J. Catalytic and chemical competence of regulation of cdc25 phosphatase by oxidation/reduction. *Biochemistry* **42**, 10060–10070 (2003).
148. Kar, S., Wang, M., Ham, S. W. & Carr, B. I. Fluorinated Cpd 5, a pure arylating K-vitamin derivative, inhibits human hepatoma cell growth by inhibiting Cdc25 and activating MAPK. *Biochem. Pharmacol.* **72**, 1217–1227 (2006).
149. Mondesert, O. *et al.* A fission yeast strain expressing human CDC25A phosphatase: a tool for selectivity studies of pharmacological inhibitors of CDC25. *Curr. Genet.* **45**, 283–288 (2004).
150. Brezak, M. C. *et al.* A novel synthetic inhibitor of CDC25 phosphatases: BN82002. *Cancer Res.* **64**, 3320–3325 (2004).
151. Kar, S., Wang, M., Yao, W., Michejda, C. J. & Carr, B. I. PM-20, a novel inhibitor of Cdc25A, induces extracellular signal-regulated kinase 1/2 phosphorylation and inhibits hepatocellular carcinoma growth *in vitro* and *in vivo*. *Mol. Cancer Ther.* **5**, 1511–1519 (2006).
152. Rudolph, J. Inhibiting transient protein-protein interactions: lessons from the Cdc25 protein tyrosine phosphatases. *Nature Rev. Cancer* **7**, 202–211 (2007).
153. Cazales, M. *et al.* Pharmacologic inhibition of CDC25 phosphatases impairs interphase microtubule dynamics and mitotic spindle assembly. *Mol. Cancer Ther.* **6**, 318–325 (2007).
154. Cheon, H. G., Kim, S. M., Yang, S. D., Ha, J. D. & Choi, J. K. Discovery of a novel protein tyrosine phosphatase-1B inhibitor, KR61639: potential development as an antihyperglycemic agent. *Eur. J. Pharmacol.* **485**, 333–339 (2004).
155. Collins, I. & Workman, P. New approaches to molecular cancer therapeutics. *Nature Chem. Biol.* **2**, 689–700 (2006).
156. Chen, Z. *et al.* Selective Chk1 inhibitors differentially sensitize p53-deficient cancer cells to cancer therapeutics. *Int. J. Cancer* **119**, 2784–2794 (2006).
157. Syljuasen, R. G. *et al.* Inhibition of human Chk1 causes increased initiation of DNA replication, phosphorylation of ATR targets, and DNA breakage. *Mol. Cell Biol.* **25**, 3553–3562 (2005).
158. Matthews, D. J. *et al.* Pharmacological abrogation of S-phase checkpoint enhances the anti-tumor activity of gemcitabine *in vivo*. *Cell Cycle* **6**, 104–110 (2007).
159. Bao, S. *et al.* Glioma stem cells promote radioresistance by preferential activation of the DNA damage response. *Nature* **444**, 687–688 (2006).
160. Thompson, J. D., Higgins, D. G. & Gibson, T. J. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res.* **22**, 4673–4680 (1994).
161. Retief, J. D. Phylogenetic analysis using PHYLIP. *Methods Mol. Biol.* **132**, 243–258 (2000).
162. van Vugt, M. A., Bras, A. & Medema, R. H. Restarting the cell cycle when the checkpoint comes to a halt. *Cancer Res.* **65**, 7037–7040 (2005).
163. Broggini, M. *et al.* Cell cycle-related phosphatases CDC25A and B expression correlates with survival in ovarian cancer patients. *Anticancer Res.* **20**, 4835–4840. (2000).
164. Kieffer, I., Lorenzo, C., Dozier, C. & Ducommun, B. Differential mitotic degradation of the CDC25B variants. *Oncogene* 2007 (in the press).

Acknowledgements
We gratefully acknowledge J. Hyams (Massey University, NZ) and J. Lazo (Pittsburgh University, USA) for critical reading of this review. We thank G. Prevost and the IPSEN group for communication on the IRC083864 compound before publication. Work in our laboratory is supported by the CNRS (Centre National de la Recherche Sicientifique), the University of Toulouse and la Ligue Nationale Contre le Cancer (Equipe Labelisée 2005). R.B. is the recipient of a C.J. Martin Fellowship (National Health and Medical Research Council; Australia) and V.L. is a post-doctoral fellow with la Ligue Nationale Contre le Cancer.

Competing interests statement
The authors declare no competing financial interests.

DATABASES
The following terms in this article are linked online to:
Entrez Gene: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene
ATM | ATR | CAK | CD133 | CDC25A | CDC25B | CDC25C | CDK1 | CDK2 | CHK1 | CTAK1 | CUL1 | E2F | HRAS | MAPKAPK2 | MELK | MKP1 | MYC | MYT1 | NMYC | p27 | p53 | PLK1 | PTP1B | RB1 | SKP1 | VHR | WEE1
Access to this links box is available online.