The regulatory ß-subunit of protein kinase CK2 accelerates the degradation of CDC25A phosphatase through the checkpoint kinase Chk1

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Abstract. Human CDC25 phosphatases play an important role in cell cycle regulation by removing inhibitory phosphate groups on cyclin-CDKs. Chk1 has been shown to phosphorylate CDC25 family members down-regulating their phosphatase activity through distinct mechanisms. The kinase activity of Chk1 is evident in unperturbed cells and becomes enhanced in response to DNA damage or stalled replication. We have previously shown that the activity of Chk1 is increased following interaction with the regulatory ß-subunit of protein kinase CK2. In the present study, ectopic expression of CK2ß during normal cell cycle progression is shown to enhance CDC25A degradation, and this occurs in a manner similar to that by which CDC25A is down-regulated upon activation of cellular checkpoint responses. By using RNA interference to specifically deplete cells of Chk1, we demonstrate that Chk1 mediates the down-regulation of endogenous CDC25A, which occurs upon induction of CK2ß expression. When degradation of CDC25A is induced by CK2ß during activation of the G2 checkpoint, it leads to partial dephosphorylation of Cdc2 at its inhibitory residue Tyr15. These results suggest that protein kinase CK2 is involved in cell cycle regulation and indicate the mechanism by which CDC25A turnover might be regulated by Chk1 in the absence of DNA damage.

Introduction

Protein kinase CK2 is a pleiotropic and ubiquitous Ser/Thr protein kinase which participates in a variety of cellular functions, including viability, regulation of cell cycle progression, cell differentiation and survival (reviewed in refs. 1,2). The classical view of CK2 as a stable tetrameric complex composed of two catalytic subunits (i.e. α and/or α') and two regulatory ß-subunits, has been modified in recent years by a number of studies indicating that the individual CK2 subunits have an independent function and that they are not exclusively co-localized but rather distributed within cells in an asymmetric manner (reviewed in refs. 1,2). Moreover, CK2ß has been shown to associate with several important protein kinases (3,4).

Among the many intracellular functions in which CK2 takes part, the regulation of cell cycle progression has perhaps been one of the most studied. In this respect, an essential role of CK2 has been demonstrated in the yeast *S. cerevisiae* by using temperature-sensitive mutant strains for the CK2 genes (reviewed in ref. 5). Furthermore, Toczyski *et al* (6) explored the yeast DNA damage checkpoint regulation and identified two adaptation-defective mutant strains that remained permanently arrested upon induction of dsDNA break underlining the requirement of CK2ß for the adaptation to G2 checkpoint in budding yeast. Similarly, studies performed employing mammalian cell lines suggested that the overexpression of CK2ß leads to attenuated proliferation resulting from cellular G2-M arrest (7). More recently, we have demostrated that CK2ß interacts with and up-regulates the checkpoint kinase Chk1 with respect to CDC25C phosphorylation (8).

The progression of the cell cycle is tightly regulated by a plethora of proteins in order to preserve genomic integrity in unperturbed cells as well as in cells exposed to DNA-damaging agents. Important mediators of these central pathways are the Ser/Thr kinases Chk1 and Chk2 (reviewed in ref. 9). Chk1 was first identified in *S. pombe* as an essential protein kinase for DNA damage-induced G2 arrest (10). Chk1 is an active kinase in unperturbed cells and its activity is further enhanced in response to DNA damage or stalled replication (reviewed in ref. 11). One of the key effectors of the G2 checkpoint is the Cdc2 kinase. Maximal activation of Cdc2 kinase requires a series of phosphorylation and dephosphorylation events and the association with cyclin B for a proper initiation of mitosis. Essential for the progression from G2 to M phase of the cell cycle is the removal of inhibitory phosphorylation from Cdc2 by a family of protein phosphatases (i.e. CDC25A, -B and -C) that are subjected to regulation by the checkpoint kinases (12-14). Previous studies have implicated hChk1 in the regulation of CDC25A stability (15,16). Chk1 phosphorylates CDC25A promoting the physiological turnover of the latter during unperturbed cell cycle progression (reviewed in ref. 17). This Chk1-mediated event is also required for an

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accelerated CDC25A turnover that follows a DNA damage-induced response.

While the essential role of Chk1 in DNA damage response has been well established, the regulation of Chk1 in normal cell cycle progression is still under investigation (18). In this respect, we have analyzed the consequences of CK2ß over-expression on the stability of CDC25 phosphatases. We provide *in vivo* evidence that CK2ß subunit is able to accelerate the degradation of CDC25A and -B in the absence of DNA damage. We also show that Chk1 mediates this effect since the cellular depletion of Chk1 by RNA interference reduces CDC25A degradation. These findings suggest that the regulatory ß-subunit of CK2 is required for Chk1 kinase activity, an essential function for the regular physiological turnover of CDC25 phosphatases in normal cell cycle progression.

Materials and methods

Cell lines and treatments. H1299 cell line was cultured in Roswell Park Memorial Institute medium (RPMI, Gibco, Taastrup, Denmark) supplemented with 10% FBS and 1 mM L-glutamine. Cells were cultured at 37˚C with 5% CO_2. Synchronization of H1299 cells was performed by a double thymidine block. Cells were treated with 2 mM thymidine (Sigma, Brondby, Denmark) for 16 h. They were then washed with phosphate-buffered saline (PBS) and released from the block by re-plating in culture medium for 9 h. A further treatment with 2 mM thymidine followed (second thymidine block) for an additional 16 h. Cells were then released from the second block by extensive washing with PBS and incubated in fresh culture medium. Where indicated, cells were transfected using FuGene 6 reagent (Roche, Penzberg, Germany) following the manufacturer's recommendations. Unless stated otherwise, 24 h after transfection cells were treated with 500 nM doxorubicin (Calbiochem, Nottingham, UK) for 24 h. For the inhibition of protein synthesis, cells were incubated with 80 μg/ml cycloheximide (CHX, Sigma) for the indicated times 36 h after transfection. Where indicated, pharmacological inhibition of Chk1 kinase was performed by incubating the cells with 100 nM Gö6976 (Calbiochem).

siRNA experiments. Chk1 expression was silenced by transfecting cells with the following oligonucleotide sequences: 5'-UCGUGAGCGUUUGUUGAACdTdT-3' (Chk1 siRNA) and 5'-GAAGCAGUCGCAGUGAAGAdTdT-3' (control siRNA), respectively, with oligofectamine (Invitrogen, Paisley, UK) according to the manufacturer's recommendations. The expression of CK2ß was silenced with a set of four siRNA duplexes directed against CK2ß-mRNA (ON-TARGET plus SMARTpool). All siRNAs were purchased from Dharmacon (Lafayette, CO, USA). Where indicated, the treatment of cells with siRNAs was followed by a second transfection 24 h later with CK2ß-MycHis expression plasmid by FuGene 6 reagent. Cells were analysed 36 h after the second transfection.

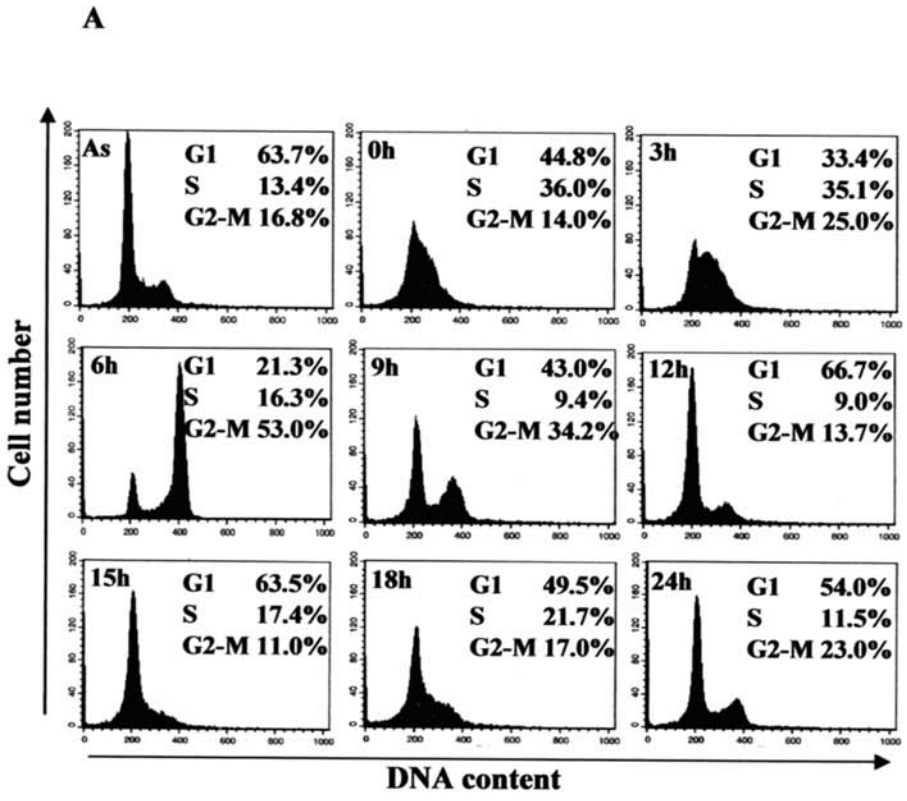
Cell cycle analysis. The cell cycle was analyzed by a FACS-Calibur flow cytometer (Becton-Dickinson, San Jose, CA). Data were analyzed by Cell Quest Pro Analysis software. Prior to analysis, cells were collected, washed with PBS and subsequently fixed in 70% ethanol overnight at -20˚C. Fixed cells were then incubated for 30 min with 20 μg/ml propidium iodide (Sigma) and 40 μg/ml RNAse (Roche) in PBS prior to analysis.

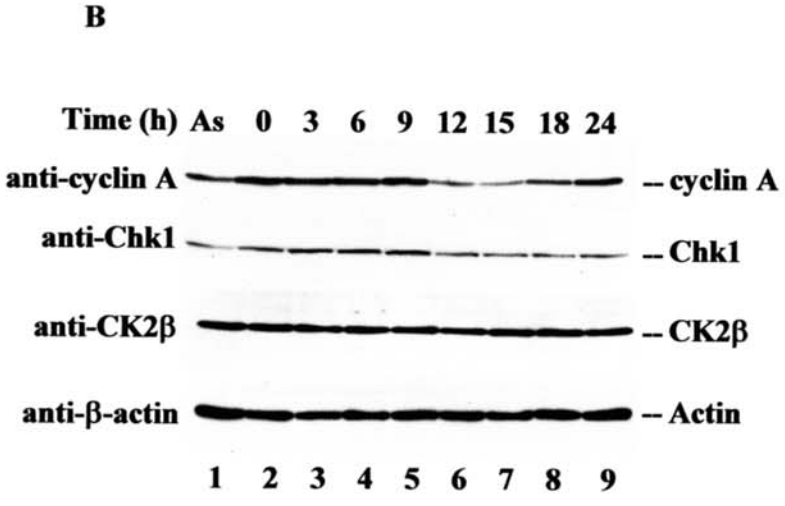
Immunoprecipitation, antibodies and Western blot analysis. Immunoprecipitation was performed essentially as previously described (8) with rabbit polyclonal anti-CK2ß serum obtained by immunizing rabbits with the full-length protein. Cell pellets were lysed in lysis buffer (50 mM Tris/HCl pH 8.5, 150 mM NaCl, 2 mM DTT, 1 mM Na_3VO_4, 100 nM okadaic acid) containing 1X protease inhibitor cocktail (Roche), sonicated and cleared by centrifugation. Proteins were separated by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and afterwards transferred to PVDF membrane (BioRad, Herlev, Denmark). PVDF membranes were probed for the indicated proteins using specific antibodies. Protein-antibody complexes were visualized by a chemiluminescence Western blot detection system according to the manufacturer's instructions (CDP-Star, Applied Biosystems, Foster City, CA, USA). Antibodies used were the following: mouse monoclonal anti-Chk1, mouse monoclonal anti-CDC25A, mouse monoclonal anti-Cdc2 p34 and rabbit polyclonal anti-cyclin A (all from Santa Cruz Biotechnology, Santa Cruz, CA, USA); mouse monoclonal anti-CK2ß and mouse monoclonal anti-CDC25B (both from Calbiochem); rabbit polyclonal anti-CDC25C and rabbit polyclonal anti-phospho-Cdc2(Tyr15) (both from Cell Signaling Technology, Beverly, MA, USA); and mouse monoclonal anti-ß-actin (Sigma). Quantification of the protein bands revealed by Western blotting was performed with Gelwork 1D Intermediate software.

Plasmid constructs. Human HA-CDC25A plasmid was kindly provided by Dr Jiri Bartek (Copenhagen, DK). The constructs encoding for full-length CK2ß (CK2ß-MycHis) and full-length Chk1 (HA-Chk1) were as described previously (8). The kinase-inactive mutant of Chk1 (HA-Chk1KD) was generated as described previously (19). Human CDC25C-MycHis was generated by the polymerase chain reaction (PCR) using the following primers: 5'-CCCAAGCTTGATGTCTACGGACT CTTCTCA-3' (sense) and 5'-CCGCTCGAGCTGGGCTCAT GTCCTTCACCAG-3' (antisense). The amplified sequence was subcloned into pcDNA 3.1 vector (Invitrogen). Human CDC25B-MycHis was generated by PCR with the following primers: 5'-CGGGATCCATGGAGGTGCCCCAGC-3' (sense) and 5'-CGCTCGAGCTGGTCCTGCAGCCG-3' (antisense) and subcloned into pcDNA 3.1 vector. In all constructs, the correct sequence and orientation were verified by DNA sequencing.

Results

Analysis of Chk1-CK2ß complex formation. Previously, we have shown that the regulatory ß-subunit of protein kinase CK2 interacts with the checkpoint kinase Chk1 and that the complex formation leads to enhanced Chk1 kinase activity (8). The aforementioned results prompted us to address the following questions: is the association between CK2ß and Chk1 confined to a particular phase of the cell cycle or independent from it; and what is the physiological relevance of Chk1-CK2ß association and hence the up-regulation of Chk1 in complex





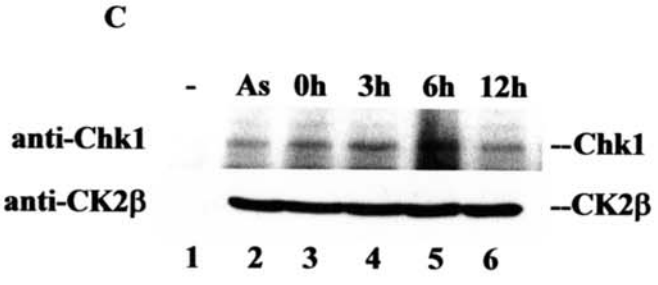


Figure 1. Analysis of cell synchronization and Chk1-CK2ß complex formation. (A) H1299 cells were synchronized at the G1-S phase by a double thymidine block method as described in the Materials and methods. The analysis of cell cycle profile was performed by FACS at the release from the second block (0 h) and at 3, 6, 9, 12, 15, 18 and 24 h after release from the second thymidine block, respectively. The cell cycle distribution of asynchronous cells is also shown (As). The percentage of cells in each phase of the cell cycle relative to the indicated time points is shown. The gain was adjusted so that cells in the G1 phase would have a fluorescence value of 200 and cells in the G2 phase a value of 400. (B) Cell extracts from synchronized cells were analyzed by probing the Western blot membrane with the indicated antibodies. (C) Lysates from asynchronous cells (As, lane 2) collected at 0, 3, 6 and 12 h (lanes 3-5), respectively, were subjected to immunoprecipitation with rabbit polyclonal anti-CK2ß antibody. Lane 1 represents a control experiment (-) where cell extract from asynchronous cells was subjected to immunoprecipitation with control serum. Chk1-CK2ß complexes were revealed by probing the Western blot membrane with monoclonal anti-Chk1 and anti-CK2ß antibodies, respectively.

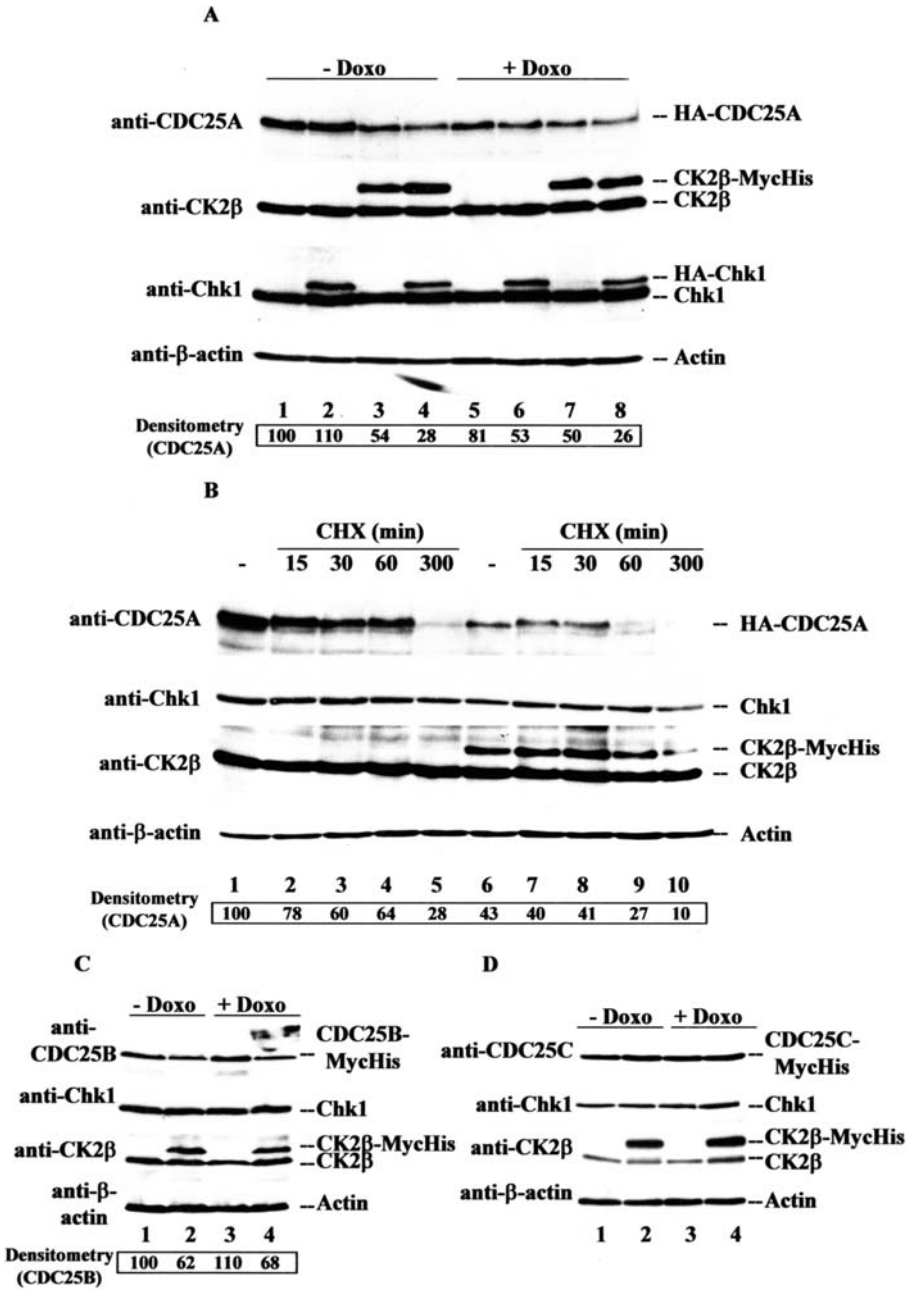


Figure 2. Ectopic expression of CK2ß leads to down-regulation of CDC25A and -B phosphatases. (A) H1299 cells were transfected with HA-CDC25A (lanes 1-8), HA-Chk1 (lanes 2, 4, 6 and 8) and CK2ß-MycHis (lanes 3, 4, 7 and 8) plasmids, respectively. Cells were left untreated (lanes 1-4) or, 24 h after transfection, incubated with 500 nM doxorubicin (lanes 5-8) for an additional 24 h. (B) H1299 cells were transfected with HA-CDC25A (lanes 1-10) and CK2ß-MycHis (lanes 6-10) plasmids, respectively. Thirty-six hours after transfection, the cells were treated with 80 μg/ml cycloheximide (CHX) for the indicated times (lanes 2-5, 7-10). (C) H1299 cells were transfected with CDC25B-MycHis (lanes 1-4) and CK2ß-MycHis (lanes 2 and 4) plasmids. Twenty-four hours after transfection cells were left untreated or incubated with doxorubicin as described in A (lanes 3 and 4). (D) The experiment was as in C except that cells expressed CDC25C-MycHis phosphatase (lanes 1-4). In all the experiments protein analysis was performed on cell extracts by Western blotting. Essentially, the same results were obtained in three independent experiments. The intensity of the bands (densitometry) on the immunoblots was quantitated and the values expressed in percentage are reported below the lane numbers.

with CK2ß in unperturbed cells as well as after induction of DNA damage? To examine the distribution of Chk1-CK2ß complexes at the different phases of the cell cycle, we carried out synchronization experiments employing H1299 cells. Cells synchronized at the G1-S border (Fig. 1A, 0h) proceeded toward the S phase of the cell cycle within 3 h after release from the block (Fig. 1A, 3h) while they moved towards the G2-M phase within 6 h (Fig. 1A, 6h). Most of the cells were in the G1 phase 12-15 h after release from the second block (Fig. 1A, 12h and 15h). The expression levels of Chk1 and CK2ß proteins were analyzed by Western blotting at various time points (Fig. 1B). Under our experimental conditions, we did not observe any variation in the expression profile of CK2ß while the expression of Chk1 gradually increased as cells entered the S phase and proceeded towards the G2-M phase (lanes 3-5) and then decreased again as in asynchronous cells (lane 1) when cells next entered the G1 phase (lanes 6-9). As a control, Fig. 1B shows the detection of cyclin A protein where the accumulation of cyclin A as cells proceed through the G1 and S phases reaching maximal levels in the G2-M phase,

supports the analysis performed by flow cytometry of cell synchronization. Cell lysates from synchronized cells collected at various time points were subjected to immunoprecipitation with polyclonal anti-CK2ß antibody. Results shown in Fig. 1C indicate that Chk1 forms stable complexes with the regulatory ß-subunit of CK2. Under our experimental conditions we did not observe any significant variation in the Chk1-CK2ß complex formation throughout the cell cycle.

Ectopic expression of the CK2 ß-subunit accelerates the degradation of CDC25A in the absence of genotoxic stress. Downstream targets of active Chk1 are members of the CDC25 family of phosphatases. In order to determine whether the ectopic expression of CK2ß has an effect on ectopically expressed CDC25 phosphatases with respect to stability and/or phosphorylation level, we examined the cell lysates from H1299 cells by Western blotting before and after induction of DNA damage (Fig. 2). DNA damage was induced by incubating cells with doxorubicin (Doxo) a topoisomerase II poison known to induce G2 DNA damage checkpoint mediated by Chk1 kinase (20). The transient expression of Chk1 did not influence the expression level of CDC25A with respect to the control experiment (Fig. 2A, lanes 1 and 2). Unexpectedly, we noticed that the expression of CK2ß was sufficient to induce a marked down-regulation of CDC25A protein level by 46% in the absence of DNA damage (Fig. 2A, lane 3). When both Chk1 and CK2ß were overexpressed the CDC25A protein level was 28% of the control (Fig. 2A, lane 4). A similar analysis was performed after exposure of cells to doxorubicin (Fig. 2A, lanes 5-8). Doxorubicin treatment caused a higher reduction in CDC25A protein levels as a consequence of Chk1-mediated degradation in response to DNA damage (20,21). To determine whether the reduction in CDC25A occurred through a decreased rate of synthesis or increased rate of degradation, we measured the half-life of the aforementioned phosphatase after cell incubation with cycloheximide (CHX) which blocks new protein synthesis (Fig. 2B). We observed that ectopically expressed CDC25A declined rapidly after 60 min (lanes 1-5). However, when cells co-expressed CDC25A and CK2ß (lanes 6-10), the CDC25A protein level appeared to be significantly low already in untreated cells (lane 6) and declined rapidly after 30 min of incubation with CHX. These results led to the conclusion that the overexpression of CK2ß accelerates the rate of degradation of ectopically expressed CDC25A phosphatase.

Next, we analyzed the expression level of CDC25B in cells treated as indicated in Fig. 2C. Exposure of H1299 cells to genotoxic stress did not result in a decline of CDC25B protein as compared with untreated cells [Fig. 2C, lanes 1 and 3; (15)]. Interestingly, we observed a reduced level of CDC25B upon induction of CK2ß overexpression in untreated as well as in cells exposed to doxorubicin (Fig. 2C, lanes 2 and 4). Fig. 2D shows the analysis of the expression level of CDC25C in the absence (lanes 1 and 3) and in the presence (lanes 2 and 4) of ectopically expressed CK2ß, respectively. Under the indicated conditions, we did not observe any variation in the expression of CDC25C protein.

Chk1 kinase activity is required for CK2ß-mediated CDC25A down-regulation. Results shown in Fig. 2 prompted us to test whether the down-regulation of CDC25A observed upon CK2ß overexpression, is mediated by Chk1 kinase. We co-expressed CDC25A phosphatase and CK2ß and we looked at the protein expression level of CDC25A from cells left untreated (Fig. 3B, lanes 1 and 3) or incubated with Gö6976, a Chk1 inhibitor (Fig. 3B, lanes 2 and 4). We performed a control experiment in order to verify whether Gö6976 was able to abrogate the Chk1-mediated G2 arrest induced by doxorubicin treatment in our model system (Fig. 3A). While the treatment with doxorubicin induced cell cycle arrest at the G2 phase after 24 h of incubation, the presence of Gö6976 in the cell medium caused abrogation of G2 arrest and subsequent cell death as reported previously (22). CDC25A (Fig. 3B, lanes 1-4) and CK2ß-MycHis (Fig. 3B, lanes 3 and 4) were transiently expressed in H1299 cells. The treatment with Gö6976 affected the overexpression of CDC25A (lane 2) as compared to untreated cells (lane 1) while the additional expression of CK2ß markedly reduced the expression of CDC25A by 67% (lane 3). However, CDC25A level was only partially reduced when the expression of CK2ß was induced in the presence of Gö6976 (lane 4). An additional experiment was performed employing a dominant-negative Chk1 mutant lacking kinase activity (Chk1KD, Fig. 3C). The sole over-expression of full-length active Chk1 (lane 2) or mutant Chk1KD (lane 3) did not affect the expression of CDC25A while the presence of ectopic CK2ß resulted in a decrease of CDC25A protein level (lane 4), which further diminished when active Chk1 (lane 5) and not the kinase-dead mutant (lane 6) were overexpressed.

CK2ß promotes degradation of endogenous CDC25A. Next, we studied whether the overexpression of CK2ß would affect the stability of endogenous CDC25A phosphatase (Fig. 4A). Incubation of cells with Gö6976 (lane 2) enhanced the exprression of endogenous CDC25A with respect to the control experiment (lane 1). Interestingly, the ectopic expression of CK2ß induced down-regulation of endogenous CDC25A phosphatase (lane 3). When CK2ß expression was induced in the presence of Gö6976 (lane 4) the degradation of CDC25A was partially rescued allowing the detection of the phosphatase. As expected, cell treatment with Doxorubicin (lane 5) down-regulated CDC25A. This result was also observed in cells overexpressing CK2ß in the presence of Doxorubicin (lane 7). Gö6976 partially rescued CDC25A from degradation induced by cell treatment with doxorubicin alone (lane 6) or in combination with CK2ß overexpression (lane 8).

Consistently, H1299 cells transfected with Chk1-directed siRNA led to a significant reduction of Chk1 protein level (Fig. 4B, lanes 2 and 3). The down-regulation of CDC25A was markedly visible only in cells expressing ectopic CK2ß and in the presence of endogenous Chk1 (Fig. 4B, lane 4). Surprisingly, when cells were transfected with CK2ß-directed siRNA (Fig. 4C, lane 2) we noticed a significant higher level of CDC25A protein with respect to the control experiment (Fig. 4C, lane 1).

Consistent with the notion that CK2ß might regulate CDC25A stability through Chk1 kinase, we tested whether the expression of CK2ß would influence the phosphorylation of Cdc2, the kinase directly responsible for the G2-M transition, in cells treated with doxorubicin for 24 h for G2 checkpoint

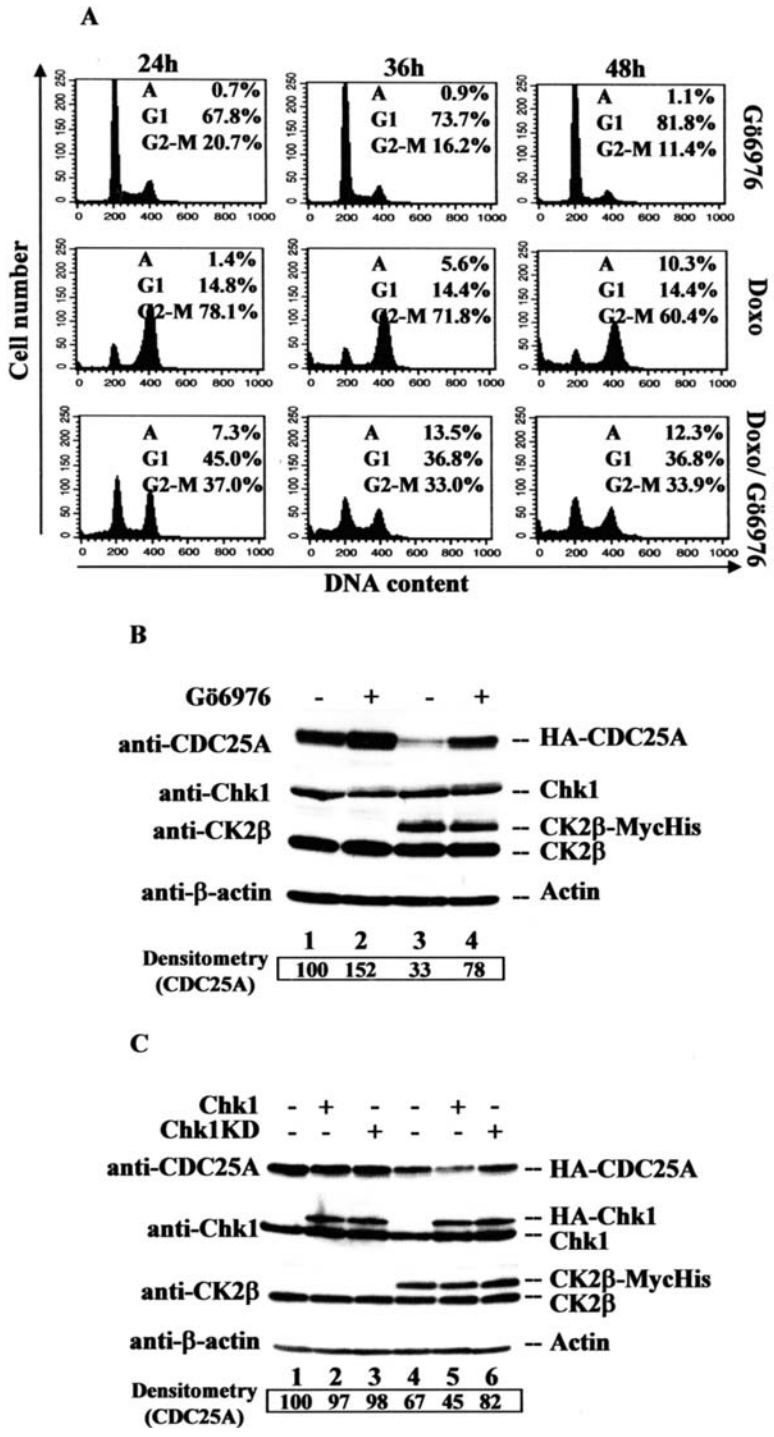


Figure 3. CK2ß-induced CDC25A protein degradation is mediated by Chk1. (A) H1299 cells were incubated with 100 nM Gö6976, 250 nM doxorubicin or a combination of Gö6976 and doxorubicin as indicated. Cells were harvested at different time points and analyzed by flow cytometry. The percentage of cells in the sub-G1, G1 and G2 phases, respectively, is shown in each panel. (B) Cells were transfected with HA-CDC25A (lanes 1-4) and CK2ß-MycHis (lanes 3 and 4) plasmids, respectively. Twenty-four hours after transfection, cells were either incubated with 100 nM Gö6976 (lanes 2 and 4) or left untreated (lanes 1 and 3) for an additional 24 h. Samples were subjected to SDS-PAGE and subsequently to Western blot analysis. Proteins were revealed by probing the membrane with the indicated antibodies. (C) H1299 cells were transfected with HA-CDC25A (lanes 1-6), HA-Chk1 (lanes 2 and 5), HA-Chk1KD (lanes 3 and 6) and CK2ß-MycHis (lanes 4-6) plasmids as indicated. Protein levels were analyzed as described in B. In all experiments, ß-actin was used as loading control. The results shown are an average of three independent experiments.

activation (Fig. 5). The overexpression of CDC25A caused dephosphorylation of Cdc2 at Tyr15 by 40% (lane 3) in agreement with a previous report (20) while the expression of CK2ß alone (lane 4) or in combination with Chk1 (lane 5), respectively, preserved the phosphorylation of Cdc2 as a consequence of enhanced degradation of CDC25A. Cdc2 protein level was not affected by the various treatments. Moreover, the overexpression of CK2ß alone caused a slight decrease in the phosphorylation level of Tyr15 (lane 2). Although speculative at the moment, this latter effect might be mediated by the CDC25B phosphatase that is reported to have a higher activity in cells overexpressing CK2 (23).

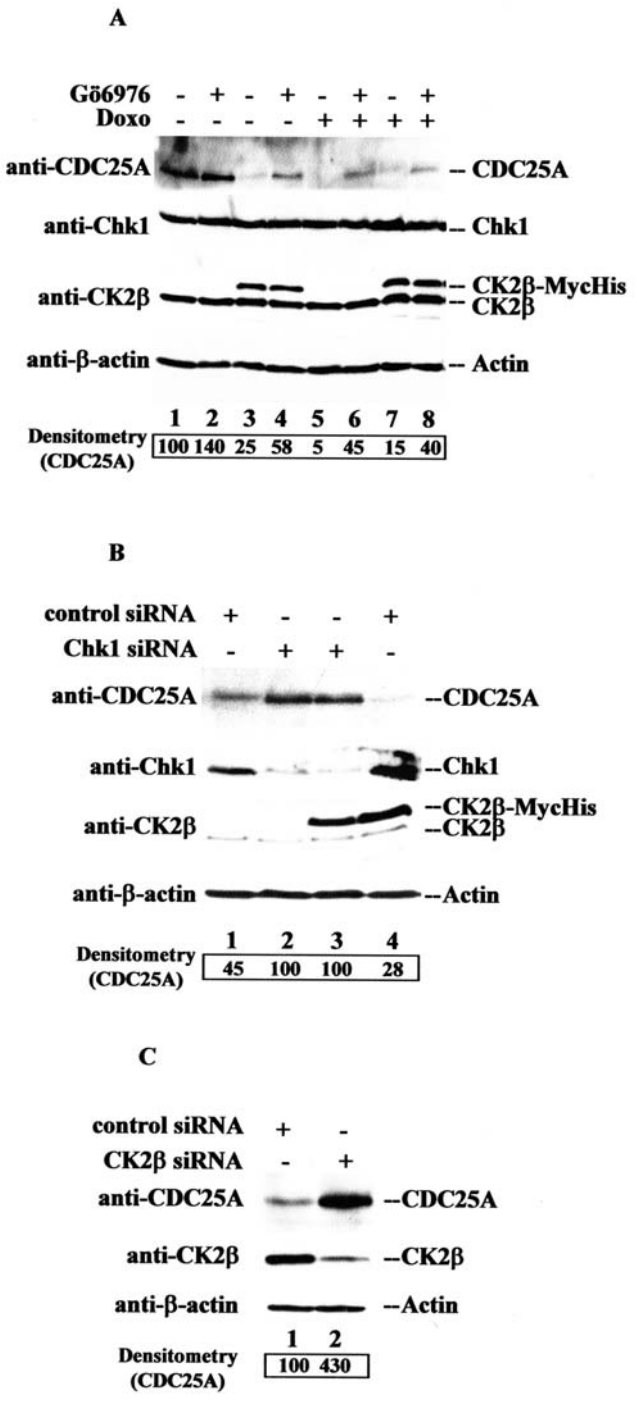


Figure 4. CK2ß accelerates the protein degradation of endogenous CDC25A. (A) H1299 cells were transfected with CK2ß-MycHis plasmid DNA (lanes 3, 4, 7 and 8). Twenty-four hours after transfection, cells were treated with 100 nM Gö6976 and 300 nM doxorubicin for an additional 24 h. The cell lysates were subjected to Western blot analysis, probing the membrane with the indicated antibodies. (B) H1299 cells were transfected with control (lane 1), Chk1 siRNAs (lanes 2 and 3) and CK2ß-MycHis plasmid (lanes 3 and 4), respectively. (C) H1299 cells were transfected with control (lane 1) or CK2ß siRNAs (lane 2). Cell lysates were analysed as described in A. The intensity of the bands on the immunoblots was quantitated and the values expressed in percentage are reported below the lane numbers. The experiments were performed four times obtaining similar results.

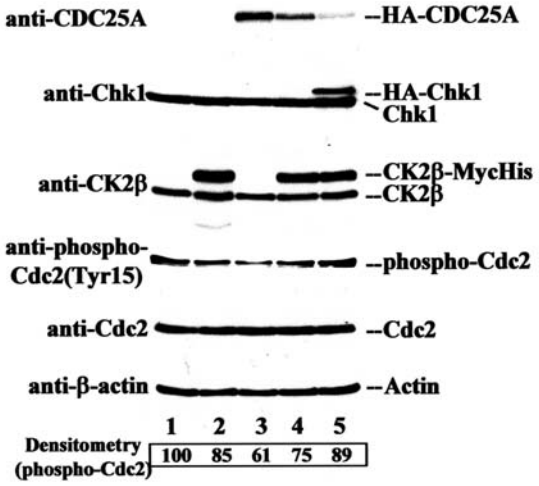


Figure 5. The overexpression of CK2ß decreases the phosphorylation of Cdc2 at Tyr15 through the down-regulation of CDC25A phosphatase. H1299 cells were transfected with HA-CDC25A (lanes 3-5), CK2ß-MycHis (lanes 2, 4 and 5) and HA-Chk1 (lane 5) plasmids. Cells were incubated with 250 nM doxorubicin for 24 h for G2 checkpoint activation. Cell lysates were analyzed by probing the Western blot membranes with the indicated antibodies.

Discussion

Cell cycle progression in higher eukaryotic cells is tightly regulated by a family of conserved protein kinases, called cyclin-dependent kinases, whose activity is determined by the binding with specific regulatory proteins (i.e. cyclins) and both positive and negative regulatory phosphorylation. During normal cell division as well as after induction of DNA damage, cells activate regulatory mechanisms, known as cell cycle checkpoints, which are central to the maintenance of genomic integrity. Chk1 is considered a major checkpoint kinase that has been shown to be responsible for the activation of the G2-M checkpoint. HChk1 exists in an active form during normal cell cycle progression and its activity is further enhanced upon induction of DNA damage. Recently, Chk1 has been shown to mediate CDC25A degradation via target phosphorylation depriving cells of an essential activator of CDKs. Moreover, it has been reported that Chk1 not only accelerates the proteolytic degradation of CDC25A after induction of DNA damage, but also regulates its basal turnover during normal cell cycle progression.

Evidence indicates that there is a close correlation between Chk1 accumulation and increase in its kinase activity irrespective of DNA damage (24). Nevertheless, one may speculate that other cellular events might contribute to Chk1 activity as it was reported that the activity of the kinase domain of human Chk1 is 20-fold more active than the full-length kinase and that the C-terminal domain plays an autoinhibitory effect on the kinase activity (25). The finding that the regulatory ß-subunit of protein kinase CK2 forms stable complexes with Chk1 suggests that CK2ß might play an important role in the regulation of Chk1 activity during normal cell cycle progression. Kinase activity might not be the only prerequisite for Chk1 functions. Chk1 interacts with components of checkpoint signalling. Thus, CK2ß might be one of the so-called 'adaptor' proteins able to recruit Chk1 to proteins of the ATM/ATR signalling cascade or to provide conditions for Chk1 phosphorylation and thus up-regulation.

In this study, we aimed to assess the *in vivo* role of CK2ß with respect to downstream targets of Chk1 (i.e. CDC25A,

-B and -C phosphatases). Our results show that in the absence of G2 checkpoint activation, the overexpression of CK2ß causes a selective acceleration of CDC25A and -B degradation, which resembles the rapid destruction of human CDC25A phosphatase induced by DNA damage (15). We have demonstrated that the down-regulation of CDC25A protein level is induced by CK2ß expression and mediated by Chk1 as the employment of a Chk1 inhibitor (i.e. Gö6976), the expression of a catalytically inactive Chk1 kinase mutant and the reduction of Chk1 protein level by RNA interference, respectively, counteract the down-regulation of CDC25A induced by CK2ß expression. The depletion of cells of CK2ß leads to a higher expression of CDC25A, which suggests that the absence of endogenous CK2ß might negatively influence the activity of Chk1. However, it cannot be excluded that other Chk1-independent mechanisms might contribute to the effects of CK2ß on CDC25A expression level. We show that CK2ß overexpression influences not only the transient expression of CDC25A but it also affects the basal turnover of endogenous CDC25A. Chk1 targets CDC25A for phosphorylation at several amino acid residues. Although speculative at the present, Chk1-CK2ß complexes might be required for the selective phosphorylation of CDC25A and thus its degradation during normal cell cycle progression. Alternatively, CK2ß might target yet unidentified components of the ubiquitylation machinery influencing positively the kinetic of this process. As in the case of CDC25A, the overexpression of CK2ß leads to lower expression of CDC25B phosphatase. Interestingly, CDC25B, which possesses a short half-life and is targeted for proteosome-dependent degradation, has also been implicated in the Chk1-mediated response to G2/M checkpoint activation (reviewed in ref. 26). The fact that the overexpression of CK2ß does not influence the stability of CDC25C comes as no surprise since CDC25C is known to be a highly stable phosphatase. Moreover, it corroborates previous studies indicating that CDC25C is not essential for G2-M progression and that CDC25A and -B may be more critical cell cycle regulators.

CDC25 phosphatase family members play a central role not only in the control of the G2-M checkpoint response to DNA damage but also in the development of cancer. CDC25A is thought to be a proto-oncogene and is overexpressed in many cancers (17,27). An attractive possibility is that the deregulated expression of CDC25A in cancer might be linked to the down-regulation of CK2ß expression, which in turn would favour the enhanced stability of CDC25A phosphatase. Although the findings reported in our study raise additional important questions, they support the notion that there might be multiple ways of regulating CDC25A turnover during normal cell division as well as checkpoint activation. Future studies should improve the knowledge on CDC25 phosphatase regulation, which is a fundamental process in the control of cell cycle progression.

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References

1. Bibby AC and Litchfield DW: The multiple personalities of the regulatory subunit of protein kinase CK2: CK2 dependent and CK2 independent roles reveal a secret identity for CK2b. Int J Biol Sci 1: 67-79, 2005.
2. Olsten ME, Weber JE and Litchfield DW: CK2 interacting proteins: emerging paradigms for CK2 regulation? Mol Cell Biochem 274: 115-124, 2005.
3. Chen M, Li D, Krebs EG and Cooper JA: The casein kinase II beta subunit binds to Mos and inhibits Mos activity. Mol Cell Biol 17: 1904-1912, 1997.
4. Hagemann C, Kalmes A, Wixler V, Wixler L, Schuster T and Rapp UR: The regulatory subunit of protein kinase CK2 is a specific A-Raf activator. FEBS Lett 403: 200-202, 1997.
5. Glover CVCIII: On the physiological role of casein kinase II in *Saccharomyces cerevisiae*. Prog Nucleic Acid Res Mol Biol 59: 95-133, 1998.
6. Toczyski DP, Galgoczy DJ and Hartwell LH: CDC5 and CKII control adaptation to the yeast DNA damage checkpoint. Cell 90: 1097-1106, 1997.
7. Li D, Dobrowolska G, Aicher LD, Chen M, Wright JH, Drueckes P, Dunphy EL, Munar ES and Krebs EG: Expression of the casein kinase 2 subunits in Chinese hamster ovary and 3T3 L1 cells provides information on the role of the enzyme in cell proliferation and the cell cycle. Biol Chem 274: 32988-32996, 1999.
8. Guerra B, Issinger OG and Wang JYJ: Modulation of human checkpoint kinase Chk1 by the regulatory beta-subunit of protein kinase CK2. Oncogene 22: 4933-4942, 2003.
9. Chen Y and Sanchez Y: Chk1 in the DNA damage response: conserved roles from yeasts to mammals. DNA Repair 3: 1025-1032, 2004.
10. Walworth N, Davey S and Beach D: Fission yeast chk1 protein kinase links the rad checkpoint pathway to cdc2. Nature 363: 368-371, 1993.
11. Bartek J and Lukas J: Chk1 and Chk2 kinases in checkpoint control and cancer. Cancer Cell 3: 421-429, 2003.
12. Krek W and Nigg EA: Differential phosphorylation of vertebrate p34cdc2 kinase at the G1/S and G2/M transitions of the cell cycle: identification of major phosphorylation sites. EMBO J 10: 305-316, 1991.
13. McGowan CH and Russell P: Human Wee1 kinase inhibits cell division by phosphorylating p34cdc2 exclusively on Tyr15. EMBO J 12: 75-85, 1993.
14. Liu F, Stanton JJ, Wu Z and Piwnica-Worms H: The human Myt1 kinase preferentially phosphorylates Cdc2 on threonine 14 and localizes to the endoplasmic reticulum and Golgi complex. Mol Cell Biol 17: 571-583, 1997.
15. Mailand N, Falck J, Lukas C, Syljuasen RG, Welcker M, Bartek J and Lukas J: Rapid destruction of human Cdc25A in response to DNA damage. Science 288: 1425-1429, 2000.
16. Zhao H, Watkins JL and 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 99: 14795-14800, 2002.
17. Busino L, Chiesa M, Draetta GF and Donzelli M: Cdc25A phosphatase: combinatorial phosphorylation, ubiquitylation and proteolysis. Oncogene 23: 2050-2056, 2004.
18. Leung-Pineda V, Ryan CE and Piwnica-Worms H: Phosphorylation of Chk1 by ATR is antagonized by a Chk1-regulated protein phosphatase 2A circuit. Mol Cell Biol 26: 7529-7538, 2006.
19. Kristensen LP, Larsen MR, Højrup P, Issinger OG and Guerra B: Phosphorylation of the regulatory beta-subunit of protein kinase CK2 by checkpoint kinase Chk1: identification of the *in vitro* CK2b phosphorylation site. FEBS Lett 569: 217-223, 2004.
20. Xiao Z, Chen Z, Gunasekera AH, Sowin TJ, Rosenberg SH, Fesik S and Zhang H: Chk1 mediates S and G2 arrests through Cdc25A degradation in response to DNA-damaging agents. J Biol Chem 278: 21767-21773, 2003.
21. Agner J, Falck J, Lukas J and Bartek J: Differential impact of diverse anticancer chemotherapeutics on the Cdc25A-degradation checkpoint pathway. Exp Cell Res 302: 162-169, 2005.

22. Kohn EA, Yoo CJ and Eastman A: The protein kinase C inhibitor Go6976 is a potent inhibitor of DNA damage-induced S and G2 cell cycle checkpoints. Cancer Res 63: 31-35, 2003.
23. Theis-Febvre N, Filhol O, Froment C, Cazales M, Cochet C, Monsarrat B, Ducommun B and Baldin V: Protein kinase CK2 regulates CDC25B phosphatase activity. Oncogene 22: 220-232, 2003.
24. Kaneko Y, Watanabe N, Morisaki H, Akita H, Fujimoto A, Tominaga K, Terasawa M, Tachibana A, Ikeda K and Nakanishi M: Cell-cycle-dependent and ATM-independent expression of human Chk1 kinase. Oncogene 18: 3673-3681, 1999.
25. Chen P, Luo C, Deng Y, Ryan K, Register J, Margosiak S, Tempczyk-Russell A, Nguyen B, Myers P, Lundgren K, Kan CC and O'Connor PM: The 1.7 Å crystal structure of human cell cycle checkpoint kinase Chk1: implications for Chk1 regulation. Cell 100: 681-692, 2000.
26. Boutros R, Dozier C and Ducommun B: The when and wheres of CDC25 phosphatases. Curr Opin Cell Biol 18: 185-191, 2006.
27. Galaktionov K, Lee AK, Eckstein J, Draetta G, Meckler J, Loda M and Beach D: CDC25 phosphatases as potential human oncogenes. Science 269: 1575-1577, 1995.