CDC25B Involvement in the Centrosome Duplication Cycle and in Microtubule Nucleation

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Abstract

Centrosome amplification is frequently reported in human cancers, although the molecular mechanisms that are responsible for this remain unclear. There is significant evidence to support a role for cyclin-dependent kinase (CDK)–cyclin complexes in centrosome duplication. The activities of CDK-cyclin complexes are, in turn, regulated by the CDC25 family of phosphatases in a strict spatiotemporal manner, and we have recently reported that CDC25B localizes to the centrosomes from early S phase. In the present study, we have investigated the role of centrosomally localized CDC25B in centrosome duplication. We first observed that overexpression of CDC25B under an inducible promoter in S phase results in centrosome overduplication. We found that forced expression of wild-type but not phosphatase-inactive CDC25B at the centrosomes results in centrosome amplification, aberrant microtubule organization, and abnormal accumulation of γ-tubulin. In contrast, inhibition of CDC25B phosphatase activity inhibits the assembly of interphase microtubules and the centrosomal localization of γ-tubulin. We propose that CDC25B is part of the pathway that controls the localization of γ-tubulin to the centrosomes, thereby regulating centrosome duplication during S phase and the nucleation of microtubules. We speculate that abnormal expression of CDC25B in numerous human tumors might therefore have a critical role in centrosome amplification and genomic instability. [Cancer Res 2007;67(24):11557–64]

Introduction

Centrosome amplification is a frequent event in almost all types of tumors and is often associated with aneuploidy and tumor aggression (1, 2). Centrosome amplification may result either from uncontrolled centrosome duplication or from cytokinetic failure (3, 4). The centrosome functions essentially to maintain cell polarity during interphase and ensure bipolar spindle assembly in mitosis (5, 6), and the components required for these activities are primarily comprised of γ-tubulin, which, in association with additional proteins, forms γ-tubulin ring complexes (γTuRC) that act as templates for microtubule nucleation (5, 7). Centrosomes have also been reported to have key roles in regulating other cell cycle events including G1 to S phase progression and exit from cytokinesis (8, 9).

Note: Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).

There is significant evidence that a specific regulatory mechanism exists to ensure synchrony between centrosome duplication, DNA replication, and cell cycle progression, and that it involves cyclin-dependent kinase 2 (CDK2; ref. 10). CDK2, in association with cyclin E or cyclin A, is essential for the initiation of both DNA replication and centriole duplication at the G1-S boundary (11). In several model systems in which prolonged G1-S phase arrest supports repeated rounds of centrosome duplication, specific inhibition or activation of CDK2-cyclin E or CDK2-cyclin A complexes could suppress or enhance repeated centrosome duplication, respectively (11–14). Centrosome duplication was further found to specifically require CDK2 and not CDK1 activity (12, 13). However, the exact mechanisms regulating the activity of CDK2 itself remain unclear.

CDK-cyclin complexes are regulated by the opposing activities of the inhibitory Wee1/Myc 1 kinases and the activatory CDC25 family of phosphatases to tightly control each cell cycle transition (reviewed in refs. 15, 16). Initially, CDK2-cyclin A and CDK2-cyclin E complexes were reported to be activated by CDC25A in G1-S phases, whereas CDC25B and CDC25C were reported to function during G2 and M phases, in the regulation of CDK1-cyclin B activity. However, more recent evidence indicates that all three CDC25 isoforms contribute to CDK1-cyclin B activation at the G2-M transition. CDC25B is proposed to first activate the centrosomal pool of CDK1-cyclin B responsible for the increase in microtubule nucleation that occurs as a consequence of centrosome maturation and other processes that commit the cell to mitosis. CDK1-cyclin B then translocates into the nucleus where the local pool then becomes activated by CDC25C. This is followed by the initiation of an auto-amplification loop in which fully activated CDK1-cyclin B further activates CDC25C and a stable form of CDC25A to promote mitotic progression (15).

Although the involvement of CDC25 isoforms in the G1-S transition are less clear, all three could potentially contribute to the activation of CDK2-cyclin E/cyclin A (perhaps in a manner analogous to that of CDK1-cyclin B activation at the G2-M transition). Indeed, CDC25A has been reported to both activate and be activated by CDK2-cyclin E/cyclin A complexes during G1-S phases (17–19), and whereas it has been reported to shuttle between the cytoplasm and nucleus (20), there are currently no reports of CDC25A detection at the centrosomes. CDC25B, on the other hand, which localizes to the centrosomes from at least the beginning of S phase until mitosis, is required for CDK1-cyclin B–mediated centrosome separation in G2 phase (21), and its centrosomal activity is regulated by activatory and inhibitory phosphorylations by Aurora A (22, 23) and CHK1 (24, 25) kinases, respectively. Together, these data led us to question whether the pool of CDC25B that localizes to the centrosomes of early interphase cells may be actively involved in the regulation of G1-S centrosome events.
In the present study, we report that CDC25B overexpression drives centrosome amplification, and that its activity is required for normal microtubule nucleation. We further show a requirement for CDC25B in the localization of γTuRC components to the centrosomes and propose a model whereby centrosomal CDC25B activity locally regulates CDK-cyclin complexes, which, in turn, regulate the centrosomal localization of γTuRCs required in the centrosome duplication cycle and in microtubule assembly. 

Materials and Methods

Cell culture. U2OS cells obtained from the American Type Culture Collection were maintained at 37°C in DMEM containing 10% FCS. U2OS cells expressing HA-CDC25B under the tetracycline-inducible promoter were cultured in the presence of 2 µg/mL tetracycline and synchronized by either 4 mmol/L hydroxyurea for 36 h or 2.5 mmol/L thymidine for 24 h.

RNA silencing. Double-stranded small interfering RNA (siRNA) oligomers were transfected into U2OS or HeLa cells using OligofectAMINE (Invitrogen) according to the manufacturer’s instructions. Briefly, cells were seeded into 24-well plates at a density of 30,000 cells per well or 60-mm culture dishes with 400,000 cells per plate and grown for 16 h before transfection with 60 pmol siRNA/0.5 mL for 40 to 48 h. Human CDC25B (24) or negative control siRNA duplexes were purchased from Dharmacon.

Plasmid construction. The vector expressing the COOH-terminal pericentrin/AKAP450 centrosome targeting (PACT) domain of AKAP450 (26) was a kind gift from Dr. S. Munro (Cambridge, United Kingdom). The AKAP450 insert was released from the vector by double digestion with BamHI and XhoI and cloned into the same sites of the pEGFP-CDC25B (pEGFP-wtB) vector, expressed in GM2163 (Dam−/−) bacteria, to create the pEGFP-wtB-PACT vector. The phosphatase-inactive CDC25B mutants (pEGFP-pdB or pEGFP-pdB-PACT), were cloned using the strategy of Gabrielli et al. (27). Briefly, the BplI-EcoRI fragments of pEGFP-wtB or pEGFP-wtB-PACT were replaced with the same fragment in which the essential catalytic Cys687 residue was mutated to a serine residue (Genecust). The CDC25B catalytic domain vector (pEGFP-wtBcat-PACT) was cloned by PCR amplification of a XhoI-BamHI fragment that was inserted into the same sites of the pEGFP-wtB-PACT vector.

DNA transfection. For transient transfection of U2OS cells with pEGFP plasmids, cells were plated onto glass coverslips in 100-mm tissue culture dishes at a density of 500,000 cells per plate, allowed to attach for 24 h and then treated with 4 mmol/L hydroxyurea for 36 h. Cells were then transfected with 3 µg DNA in the presence of hydroxyurea, using Lipofectin reagent (Life Technologies) according to the manufacturer’s instructions. Four hours post-transfection, cells were fixed and stained for α-tubulin or γ-tubulin for quantitation of microtubule defects and γ-tubulin intensity at the centrosomes, respectively. For quantitation of centrosome amplification, cells were incubated for a further 6 h in the absence of hydroxyurea to allow S-phase progression and then fixed and stained for γ-tubulin.

Immunofluorescence microscopy. For immunofluorescence studies, U2OS cells seeded onto glass coverslips in 100-mm tissue culture dishes at a density of 500,000 cells per plate, were allowed to attach for 24 h and then treated with 4 mmol/L hydroxyurea for 36 h. Cells were then transfected with 3 µg DNA in the presence of hydroxyurea, using Lipofectin reagent (Life Technologies) according to the manufacturer’s instructions. Four hours post-transfection, cells were fixed and stained for α-tubulin or γ-tubulin for quantitation of microtubule defects and γ-tubulin intensity at the centrosomes, respectively.

Western blot analyses. Cell pellets were lysed into buffer containing 50 mmol/L Tris (pH 7.5), 150 mmol/L sodium chloride, 1% deoxycholic acid, 1% SDS, 1% NP40, 1 mmol/L DTT, and phosphatase and protease inhibitors, and sonicated. For partitioning into soluble and insoluble protein fractions, soluble proteins were isolated in a buffer containing 10 mmol/L sodium phosphate (pH 7.4), 150 mmol/L NaCl, 1% NP40, and 0.1% SDS with inhibitors. Lysates were centrifuged, and the insoluble pellet resuspended in buffer containing 125 mmol/L Tris-HCl (pH 6.8), 3% SDS, and 5% β-mercaptoethanol with inhibitors. Proteins were separated on 4% to 12% bis-tris gels and transferred onto nitrocellulose membranes. Anti-CDC25B (C-20), CDK2 (H-298), CDK1 (17), and cyclin B1 (GNS1) were from Santa Cruz Biotechnology, anti-cyclin A was from Becton Dickinson, anti-γ-tubulin (GTU-88) and α-tubulin (B-5-1-2) monoclonal antibodies were from Sigma-Aldrich, anti-HA monoclonal antibody was from Babco, anti-green fluorescent protein (anti-GFP) was from Roche, and anti-actin was from Chemicon. Secondary antibodies labeled with horseradish peroxidase were purchased from Cell Signaling Technology Inc.

Fluorescence-activated cell sorting analyses. To determine cell cycle profiles of U2OS cells stably expressing HA-CDC25B under the tetracycline-inducible promoter, cells were harvested and fixed in ice-cold 70% ethanol, washed with PBS containing 1% bovine serum albumin, and permeabilized with 0.25% Triton X-100 for 7 min. Cells were stained with an anti-HA monoclonal antibody (Babco), followed by a secondary antibody labeled with Alexa-488 (Invitrogen). DNA was labeled with propidium iodide (10 µg/mL) in the presence of RNase (1 mg/mL).

Results

CDC25B overexpression drives centrosome overduplication. We first examined the consequences of CDC25B overexpression on the centrosome duplication cycle. U2OS cells conditionally expressing CDC25B with a HA tag (29) were synchronized in G1-S phases using either hydroxyurea (HU) or thymidine. The expression of CDC25B was induced by the removal of tetracycline in parallel with the removal of the cell cycle synchronizing agents and allowed to proceed through S phase (Supplementary Fig. S1). Cells were harvested from 4 to 8 h post-release, and their centrosome numbers (Fig. 1) and cell cycle distributions (Supplementary Fig. S1) were analyzed by immunofluorescence and fluorescence-activated cell sorting (FACS) analyses, respectively.

Statistical and image analyses. All statistical analyses were done using paired Student’s t tests and Microsoft Excel or Prism software packages. Image analyses were done using the Metamorph imaging software package as outlined in the figure legends.
CDC25B localizes to the centrosomes of interphase cells from at least early S phase (25), suggested a possible role for CDC25B in the regulation of the centrosome cycle. We therefore examined the role of CDC25B in centrosome duplication by specific targeting of wild-type (wt) or phosphatase-dead (pd; ref. 27) forms of CDC25B to the centrosomes. We attached the PACT domain of the centrosome anchoring protein AKAP450, which includes its conserved centrosome-targeting motif (26) to the COOH-terminal ends of wt (wtB-PACT) and pd (pdB-PACT) pEGFP-CDC25B variants. To examine centrosome numbers, U2OS cells synchronized by HU treatment were transfected (in the continued presence of HU) for 4 h and then released and incubated for a further 6 h to allow progression through S phase (Fig. 2 and Supplementary Fig. S2B). Quantification of γ-tubulin–positive centrosomes in S- and G2-phase cells revealed that significant proportions of wtB- and wtB-PACT–transfected cells had more than two centrosomes (see example, Fig. 2A), and these were more frequent in cells expressing wtB-PACT (47%) than wtB (36%; P = 0.05; Fig. 2B). Cells transfected with the phosphatase-dead (pdB or pdB-PACT) vectors, on the other hand, displayed only low levels of centrosome amplification (10% and 8%, respectively), comparable with cells transfected with the control PACT vector (Fig. 2B). Western blot analyses revealed increased levels of CDK2 and cyclin E proteins in cells transfected with either the wtB or wtB-PACT vectors, but not the pdB vectors (Supplementary Fig. S2B). Together, these data support a significant role for catalytically active CDC25B in the centrosome duplication cycle.

Overexpression of phosphatase-active CDC25B at the centrosomes results in aberrant microtubule nucleation. We have recently reported that inhibition of CDC25 phosphatase activities by a brief treatment with the chemical compound BN82685 results in a complete failure of interphase microtubules to nucleate. We therefore examined the role of CDC25B in centrosome duplication by specific targeting of wild-type (wt) or phosphatase-dead (pd; ref. 27) forms of CDC25B to the centrosomes. We attached the PACT domain of the centrosome anchoring protein AKAP450, which includes its conserved centrosome-targeting motif (26) to the COOH-terminal ends of wt (wtB-PACT) and pd (pdB-PACT) pEGFP-CDC25B variants. To examine centrosome numbers, U2OS cells synchronized by HU treatment were transfected (in the continued presence of HU) for 4 h and then released and incubated for a further 6 h to allow progression through S phase (Fig. 2 and Supplementary Fig. S2B). Quantification of γ-tubulin–positive centrosomes in S- and G2-phase cells revealed that significant proportions of wtB- and wtB-PACT–transfected cells had more than two centrosomes (see example, Fig. 2A), and these were more frequent in cells expressing wtB-PACT (47%) than wtB (36%; P = 0.05; Fig. 2B). Cells transfected with the phosphatase-dead (pdB or pdB-PACT) vectors, on the other hand, displayed only low levels of centrosome amplification (10% and 8%, respectively), comparable with cells transfected with the control PACT vector (Fig. 2B). Western blot analyses revealed increased levels of CDK2 and cyclin E proteins in cells transfected with either the wtB or wtB-PACT vectors, but not the pdB vectors (Supplementary Fig. S2B). Together, these data support a significant role for catalytically active CDC25B in the centrosome duplication cycle.

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repolymerize from the centrosome after cold treatment in HeLa cells (30). This observation, together with the centrosomal localization of CDC25B (25), led us to examine, using both knock-in and knockdown approaches, whether CDC25B isoform regulates interphase microtubule dynamics.

To examine the effects of overexpressing wild-type or phosphatase-dead CDC25B on interphase microtubule nucleation, G1-S phase–arrested U2OS cells were transfected for 4 h in the presence of HU and fixed immediately and co-stained with anti–α-tubulin (Fig. 3).

Surprisingly, the majority of interphase cells transfected with either of the wild-type (wtB or wtB-PACT) vectors had well-separated centrosomes just 4 h post-transfection. Observation of the microtubules revealed that 80% of interphase cells (Fig. 3A) expressing wtB-PACT displayed very abnormal microtubule networks (as exemplified in Fig. 3B), including cells with increased microtubule nucleation from one or more centrosomes or abnormal microtubule structures within the nucleus that often formed a bridge between the centrosomes (Fig. 3B and data not shown). Similar phenotypes could be observed in cells expressing the wtB pEGFP fusion protein, although with significantly lower frequency (P = 0.01, Fig. 3A) and severity, relative to the levels of fusion proteins expressed (Fig. 3B and Supplementary Fig. S2A). In contrast, the percentages of cells transfected with the phosphatase-dead (pdB or pdB-PACT) vectors that had very minor microtubule abnormalities were similar to that of cells transfected with PACT alone (Fig. 3A and B). In addition, cells transfected with either of the pdB vectors had microtubules that were poorly or incompletely polymerized in comparison to those of PACT-transfected control cells (Fig. 3B), consistent with a negative effect of phosphatase-dead CDC25B expression on endogenous CDC25B activity and, therefore, on microtubule dynamics (27).

**CDC25B activity is required to maintain the interphase microtubule network.** To examine the effect of CDC25B inhibition on the microtubule network, U2OS (Fig. 4 and Supplementary Fig. S3) and HeLa (data not shown) cells were transfected with control or CDC25B siRNA duplexes for 48 h, which resulted in a transfection efficiency of 15% to 30% that was sufficient to reduce CDC25B protein levels (Supplementary Fig. S3) without causing significant cell toxicity. Cells were then fixed and stained for α-tubulin to mark the microtubule network and for CDC25B-S230P to detect the centrosomal associated pool of CDC25B (25). As shown in Fig. 4A, cells treated with CDC25B siRNA, in which CDC25B signal is significantly reduced or abolished from the centrosomes, display abnormal microtubule networks, comprised of poorly polymerized microtubules that seem to lack organization, in comparison to cells treated with control siRNA in which the microtubule networks were normal (Fig. 4A). Quantitation of this effect by analyses of the lengths of individual fiber fragments generated by image convolution was done as described (31) using the Metamorph software. This study revealed a significant reduction in the average lengths of individual fibers following CDC25B siRNA in comparison to control siRNA (Fig. 4B). In addition, the average number of fibers counted per cell was 1,038 in control cells (n = 15 cells) in comparison to 496 in CDC25B siRNA-treated cells (n = 22 cells), consistent with a decrease in the insoluble pool of α-tubulin by Western blot analyses (Supplementary Fig. S3). These data are consistent with those obtained from overexpression studies (Fig. 3) and our previous results (30), thereby supporting a significant role for centrosomally localized, phosphatase-active CDC25B in the nucleation of interphase microtubules.

**Centrosomal CDC25B regulates γ-tubulin recruitment to the centrosomes.** Because inhibition or overexpression of CDC25B had inverse effects on both the duplication of centrosomes and microtubule nucleation (Figs. 1–4), which have both been reported to be regulated by centrosomal accumulation of γ-tubulin (28, 32–37),...
we next observed whether the centrosomal pool of γ-tubulin was affected by CDC25B activity. As shown in Fig. 5A (left), U2OS cells successfully treated with CDC25B siRNA have significantly diminished γ-tubulin levels at the centrosomes, in comparison to surrounding unaffected cells or cells treated with control siRNA. To quantify this, we counted siRNA-treated cells in which CDC25B-S230P signal was completely diminished (CDC25B siRNA) or not affected (control siRNA) and accompanied by a substantial reduction in centrosomal γ-tubulin signal (Fig. 5A). Approximately 48% of cells treated with CDC25B siRNA had undetectable or substantially decreased γ-tubulin levels, in comparison to only 4% in cells treated with control siRNA (Fig. 5A, right), suggesting that CDC25B is required for the centrosomal recruitment of γ-tubulin.

To further examine the role of CDC25B in γ-tubulin recruitment and its associated centrosomal functions, U2OS cells were transfected with the pEGFP-PACT vectors for 4 h as described above and fixed and co-stained with anti-γ-tubulin. As shown in Fig. 5B and Supplementary Fig. S4, the centrosomal levels of γ-tubulin in cells transfected with both the wtB or wtB-PACT vectors for just 4 h were significantly increased in comparison to untransfected surrounding cells and cells transfected with PACT alone or the phosphatase-dead (pdB or pdB-PACT) vectors. Although cells transfected with pdB or pdB-PACT showed slight decreases in γ-tubulin levels in most cases, this was not statistically significant (Supplementary Fig. S4), suggesting that whereas 4 h transfection with wild-type CDC25B is sufficient to cause increased recruitment of γ-tubulin, it is not sufficient for phosphatase-dead CDC25B to completely inhibit the activity of endogenous CDC25B, as previously observed following 48 h transfection (27). In addition, although cells transfected with wtB also displayed increases in centrosomal levels of γ-tubulin in G1-S (Supplementary Fig. S4A) and G2 (Supplementary Fig. S4B) phases, this increase was more marked with wtB-PACT, particularly in cells displaying centrosome amplification (Supplementary Fig. S4C), suggesting a specific role for centrosomal phosphatase-active CDC25B in γ-tubulin recruitment and/or retention at the centrosomes during interphase.

To determine whether CDC25B may be necessary for the recruitment of other components of the γTuRC, we next examined whether the levels of centrosomal NEDD1 were similarly increased after transfection with wild-type CDC25B. As shown in Supplementary Fig. S5, the centrosomal level of NEDD1 was increased in cells transfected with wtB-PACT, in comparison to those transfected with either the PACT or pdB-PACT vectors. On the other hand, expression of centrin, a structural component of the centriole, was not obviously altered in cells transfected with any of the vectors (data not shown).

These data strongly suggest that CDC25B is active at the centrosomes from the early stages of their duplication cycle, and that its activity is required for the centrosomal localization of γTuRCs, which are, in turn, required for microtubule assembly throughout the cell cycle.

Discussion

We have recently reported that CDC25B localizes to the centrosomes during interphase (25), and in the current study, we have explored the functional significance of this localization. We report that CDC25B catalytic activity is required for the centrosomal localization of γTuRCs, for duplication of the centrosome, and for the assembly of interphase microtubules.

We first observed that U2OS cells, in which CDC25B overexpression is induced, have a high frequency of centrosome amplification. Quantification of this phenotype revealed that cells that are allowed to progress through S phase concurrent with the induction of CDC25B overexpression undergo centrosome over-duplication at frequencies up to three to four times those of control cells (Fig. 1). Significantly, this was found to be both dependent on the phosphatase activity of CDC25B and specifically mediated by centrosomally localized CDC25B (Fig. 2). Together, these data strongly support a novel role for the CDC25B phosphatase in the centrosome duplication cycle.

CDC25B regulates the activity of CDK1-cyclin B complexes at the centrosomes during the G2-M transition (21, 38), and this activity is required for the increased microtubule nucleation that occurs before the onset of mitosis (27). CDC25B therefore has an established role in the organization of the premitotic microtubule array. In the present study, we show that CDC25B is also required for the nucleation of interphase microtubules, from at least the
beginning of the S phase. Specific RNAi-mediated depletion of CDC25B resulted in the failure of interphase microtubules to assemble and to form centrosome-associated asters (Fig. 4). Overexpression of CDC25B, on the other hand, resulted in obvious defects in microtubule organization, such as increased microtubule nucleation from one or more centrosomes, the formation of microtubule bridges between the centrosomes, and the accumulation of microtubule bundles within the nucleus (Fig. 3 and data not shown) that were exacerbated by specific targeting of CDC25B overexpression to the centrosomes with the PACT domain. Importantly, these defects were not observed when the catalytically inactive form of CDC25B was used either with or without specific targeting to the centrosome (Fig. 3), indicating that CDC25B activity specifically affects microtubule assembly. In line with these findings, we have recently observed that inhibition of all CDC25 phosphatase activity with low concentrations of the chemical inhibitor BN82685 not only retards the assembly of the mitotic spindle, but also adversely affects the interphase microtubule network (30).

Because we found that CDC25B localizes to the centrosome from G1 until late G2 phases (25), we argued that centrosomal CDC25B might be involved in the recruitment of essential components required for microtubule assembly. Indeed, we found that inhibition of CDC25B resulted in a significant decrease in centrosomal γ-tubulin signal by immunofluorescence analyses, and in the inverse case, that overexpression of wild-type but not phosphatase-dead CDC25B resulted in a significant increase in the centrosomal localization of γ-tubulin and NEDD1, a γTuRC component that has been shown to be required for their recruitment to the centrosomes in interphase and mitotic cells (refs. 28, 39; Fig. 5 and Supplementary Figs. S4 and S5).

To determine whether the increased microtubule nucleation and centrosomal γ-tubulin recruitment observed with CDC25B overexpression was specific to the CDC25B isoforms or might be due to a general increase in phosphatase activity, we performed RNAi-mediated knockdown of the CDC25B isoforms S230P and S230A, which are catalytically inactive, and compared the results with those obtained with wild-type CDC25B. As shown in Figure 5A, CDC25B-S230P resulted in a significant decrease in centrosomal γ-tubulin signal by immunofluorescence analyses, and in the inverse case, that overexpression of wild-type but not phosphatase-dead CDC25B resulted in a significant increase in the centrosomal localization of γ-tubulin and NEDD1, a γTuRC component that has been shown to be required for their recruitment to the centrosomes in interphase and mitotic cells (refs. 28, 39; Fig. 5 and Supplementary Figs. S4 and S5).

Figure 5. CDC25B is required for the localization of γ-tubulin to the centrosomes. A, U2OS cells were treated with CDC25B or control siRNA duplexes for 48 h before immunofluorescent detection of CDC25B (CDC25B-S230P) and γ-tubulin. Left, examples of siRNA-treated cells; arrowheads, those in which CDC25B was successfully diminished at the centrosomes. Right, percentages of cells in which γ-tubulin and CDC25B-S230P (CDC25B siRNA only) were not detectable or substantially diminished. At least 200 cells were counted from each of three independent experiments. Columns, means; bars, SD. B, U2OS cells were synchronized and transfected with the pEGFP-CDC25B vectors as in Fig. 2 for 4 h in the presence of HU and fixed and co-stained with anti-γ-tubulin. Cells shown are examples of the intensity of centrosomal γ-tubulin signal in transfected cells, in comparison with surrounding untransfected cells, at approximately the same cell cycle stage. Insets, magnifications of the centrosome area in cells marked by an arrowhead. Bar, 10 μm.
induced by overexpression of any CDC25 phosphatase activity, we targeted the catalytic domain of CDC25B (amino acids 463–627), which is conserved between all three CDC25 isoforms to the γTuRCs to the centrosomes, control centrosome duplication and microtubule nucleation throughout the cell cycle. This would also ensure that the centrosome duplication cycle remains in synchrony with the DNA replication cycle.

Interestingly, when CDC25B was overexpressed in cells that were synchronized at the G1-S boundary, the majority of them had well-separated centrosomes or had entered into a state of mitotic catastrophe (as reported previously; refs. 27, 43), with unreplicated or incompletely condensed DNA accompanied by the formation of bi- or multipolar parastripes, as early as 4 h after transfection (data not shown). This phenotype is very interesting given the facts that overexpression of CDC25B and centrosome abnormalities are both common features of many cancers that have been associated with more aggressive characteristics (2, 16, 44). It will therefore be of major interest to examine whether increased CDC25B expression might participate in centrosome amplification and chromosomal instability during carcinogenesis.

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