

Report

Characterization of centrosomal localization and dynamics of Cdc25C phosphatase in mitosis

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Abbreviations: Cdk, cyclin-dependent kinase; FRAP, fluorescence recovery after photobleaching; MPF, mitosis promoting factor; NA, numerical aperture; siRNA, small interfering RNA

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In mammalian cells, three Cdc25 phosphatases A, B, C coordinate cell cycle progression through activating dephosphorylation of Cyclin-dependent kinases. Whereas Cdc25B is believed to trigger entry into mitosis, Cdc25C is thought to act at a later stage of mitosis and in the nucleus. We report that a fraction of Cdc25C localises to centrosomes in a cell cycle-dependent fashion, as of late S phase and throughout G₂ and mitosis. Moreover, Cdc25C colocalises with Cyclin B1 at centrosomes in G₂ and in prophase and Fluorescence Recovery after Photobleaching experiments reveal that they are both in dynamic exchange between the centrosome and the cytoplasm. The centrosomal localisation of Cdc25C is essentially mediated by its catalytic C-terminal domain, but does not require catalytic activity. In fact phosphatase-dead and substrate-binding hotspot mutants of Cdc25C accumulate at centrosomes together with phosphoTyr15-Cdk1 and behave as dominant negative forms that impair entry into mitosis. Taken together, our data suggest an unexpected function for Cdc25C at the G₂/M transition, in dephosphorylation of Cdk1. We propose that Cdc25C may participate in amplification of Cdk1-Cyclin B1 activity following initial activation by Cdc25B, and that this process is initiated at the centrosome, then further propagated throughout the cytoplasm thanks to the dynamic behavior of both Cdc25C and Cyclin B1.

Introduction

Progression through the different phases of the eukaryotic cell division cycle is a tightly regulated process that is coordinated by sequential activation of members of the family of the Cyclin-dependent kinases.¹ Mitosis in particular is orchestrated by Cdk1-Cyclin B1 kinase, also called MPF (for "Mitosis-Promoting Factor"), whose universal and central role ensures timely and coordinated chromo-

some segregation through activation of a number of substrates and establishment of the appropriate mitotic structures.^{2,3} MPF activation is a complex process, which involves a number of phosphorylation and dephosphorylation events, including phosphorylation of Cyclin B1, phosphorylation of Cdk1 on Thr¹⁶¹ by Cdk-activating kinase CAK, and dephosphorylation of phosphorylated Cdk1 on Thr¹⁴ and Tyr¹⁵ by dual specificity Cdc25 phosphatases.⁴⁻⁶ In human cells three Cdc25 isoforms, A, B and C assume overlapping functions in orchestrating cell cycle progression.⁷ Cdc25B and Cdc25C have been more directly implicated in activation of MPF.⁸⁻¹³ Active MPF in turn participates in further activation of Cdc25 phosphatases through a positive feedback loop, thereby amplifying MPF activity to a threshold level, which triggers unconditional and irreversible entry into mitosis.^{14,15}

Although it is generally accepted that entry into mitosis requires accumulation of active Cdk1-Cyclin B1 in the nucleus,^{3,16} a number of studies point to initial activation of MPF in the cytoplasm, and more recently to the centrosome,^{13,17-20} and substrates of MPF are found in both the nucleus and the cytoplasm.²¹⁻²⁴ The active form of Cdk1-Cyclin B1, in which Cyclin B1 is hyperphosphorylated, first appears at centrosomes in prophase.¹⁸ Moreover, a growing number of reports document the physical association of mitotic regulators with the centrosome, including Plk1 and Cdc25B, thereby pointing to this organelle as a centralizing platform, which coordinates the complex sequence of events leading to MPF activation.^{6,10,13,17,18,25-31} Recent work suggests a dynamic relationship between Cdc25B and Chk1 on the one hand, and Aurora-A and Eg3 on the other, in centrosomal activation of Cdc25B.^{13,27-30} The state-of-the-art model in mammalian cells implies that Cdk1-Cyclin B1 activation first occurs at centrosomes in prophase, as a consequence of Cdc25B-mediated dephosphorylation of centrosomal Cdk1. Cdc25C is sequestered by 14-3-3 proteins in the cytoplasm throughout interphase,³²⁻³⁵ and is then activated and translocated into the nucleus at the G₂/M transition following dissociation from 14-3-3 proteins and hyperphosphorylation by Plk and Cdk-Cyclin kinases.³⁶⁻⁴² Cdc25C is therefore thought to contribute to amplification of MPF activity well after the G₂/M transition, in the nucleus, where it would counteract the inhibitory function of Wee1

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kinase.^{11-13,16,36} However its biological features and functional implication at the G₂/M transition are not fully understood.

Results

Cdc25C localises to centrosomes in G₂ and during mitosis. We reinvestigated the subcellular localisation of Cdc25C in HeLa cells using an antibody raised against a specific C-terminal sequence of Cdc25C (C-20) and found, in agreement with another recent study,⁴³ that whilst most Cdc25C localises to the cytoplasm in asynchronous cells, a fraction of endogenous Cdc25C localises to centrosomes, co-localising with structures staining for pericentriolar material and γ -tubulin (Fig. 1A and C). This localisation persists throughout G₂ and mitosis, from prophase through telophase and is clearly specific, as centrosomal staining disappears upon treatment with Cdc25C siRNA, whereas neither Cdc25A nor Cdc25B levels are affected (Fig. 1B and C), or upon incubation of the antibody with its corresponding immunization peptide (Suppl. Fig. 1). The centrosomal localisation of Cdc25C was also observed in U2OS osteosarcoma and in non-transformed, non-immortalized NHF and HS68 human diploid fibroblasts, further supporting its biological relevance (data not shown). Moreover, Cdc25C co-fractionates with centrosomal γ -tubulin in centrosomal preparations purified from synchronized cells in G₂, implying it is physically associated with centrosomes (Fig. 1D).

To address whether centrosomal localisation of Cdc25C was cell cycle-dependent, we used a HeLa cell line which stably expresses centriolar GFP-centrin, and examined when Cdc25C appeared at centrosomes. We found that Cdc25C first appears at duplicating centrosomes and further accumulates when centrosomes are fully duplicated and begin to separate, in G₂ (Fig. 2A and B). To further assess at which stage endogenous Cdc25C localizes to centrosomes, we compared the kinetics of centrosomal Cdc25C accumulation with those of Cyclin A, which revealed a general trend indicative of initial appearance of Cdc25C at centrosomes as of late S phase, then accumulating throughout G₂ (Fig. 2C and D).

Cdc25C and Cyclin B1 colocalise at centrosomes in G₂ and in prophase and are highly dynamic. In mammalian cells Cyclin B1 accumulates at centrosomes in G₂, and is first activated and hyperphosphorylated at this organelle in prophase.¹⁸ Indirect immunofluorescence performed on HeLa cells revealed that the endogenous

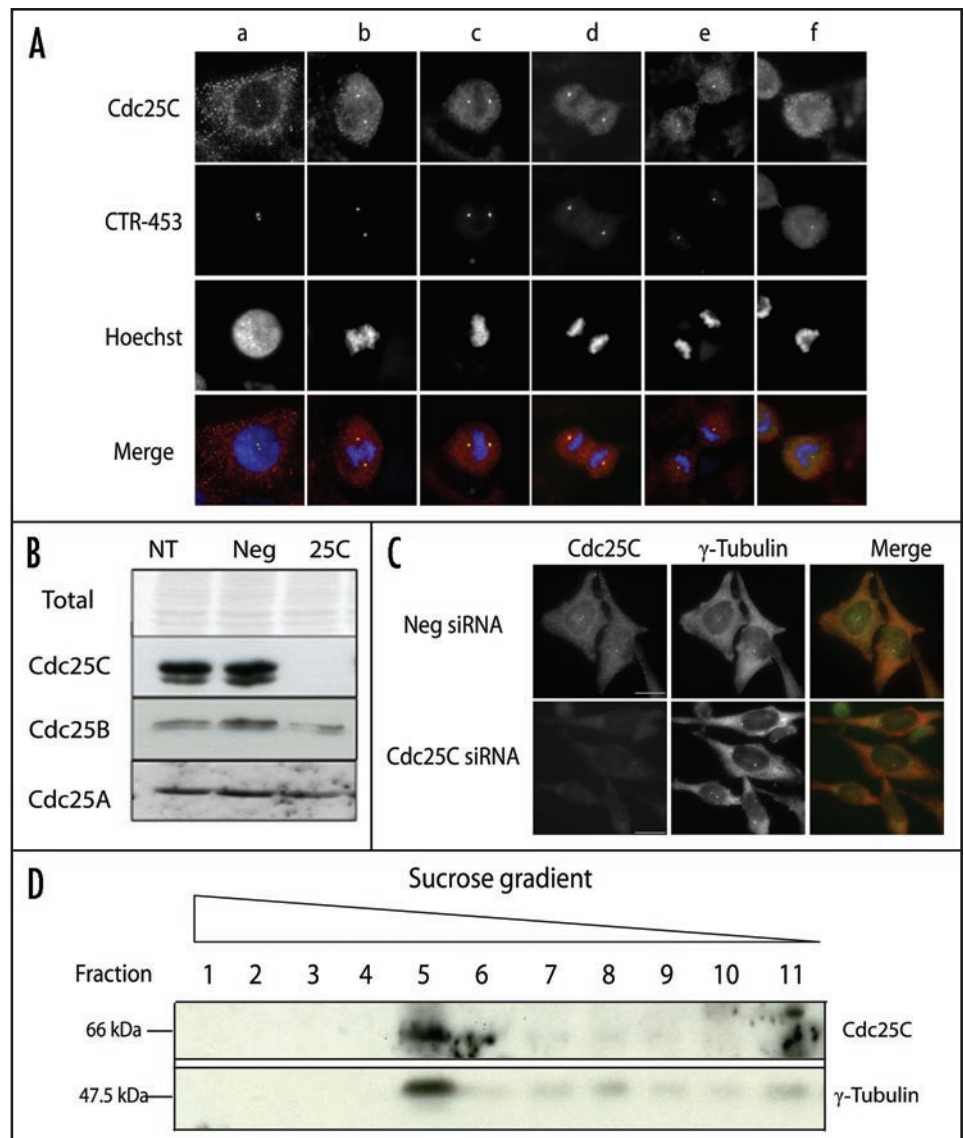


Figure 1. Cdc25C localises to centrosomes in G₂ and mitosis. (A) Indirect immunofluorescence of endogenous Cdc25C with the C-20 antibody. Cells in prophase (A), prometaphase (B), metaphase (C), anaphase (D), telophase (E) and cytokinesis (F). Centrosomes were stained with the anti-CTR453 antibody, DNA with Hoechst 33342. Bar: 10 μ m. Images were acquired with a 63X objective. (B) Western blot with anti-Cdc25A, B or C, of cell extracts prepared from untreated cells (NT) or cells treated with control siRNA (Neg) or siRNA targeting Cdc25C (25C). (C) Indirect immunofluorescence performed with anti-Cdc25C C-20 and anti-gamma-tubulin antibodies on HeLa cells treated with siRNA targeting Cdc25C (lower) or control siRNA (upper). Bar: 20 μ m. Images were acquired with a 40X objective. (D) Western blot with anti-Cdc25C and anti-gamma-tubulin antibodies of centrosomal fractions purified on a sucrose gradient.

form of Cdc25C colocalised with Cyclin B1 both at centrosomes and in the cytoplasm in G₂ and in prophase (Fig. 3A). Likewise, ectopically expressed mRFP-Cdc25C and EGFP-Cyclin B1 colocalised in both the cytoplasm and at centrosomes (Fig. 3B). EGFP-tagged Cyclin B1 and Cdc25C (localisation of which is identical to that of mRFP-Cdc25C) were microinjected into HeLa cells in G₂ and fluorescence recovery after photobleaching (FRAP) experiments were performed by photobleaching the centrosomal fraction of these proteins, to characterise their mobility (Fig. 3C and D). These studies revealed that centrosomal EGFP-Cdc25C was highly dynamic, the

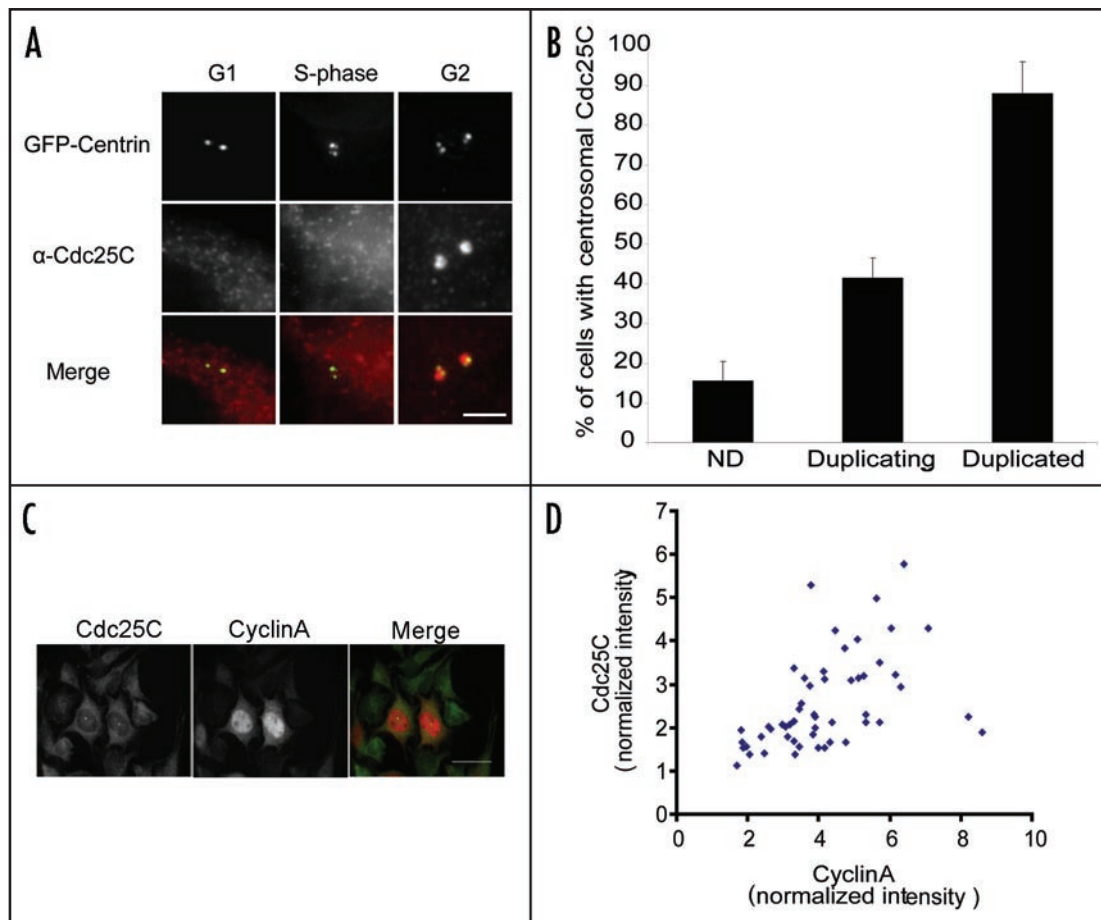


Figure 2. Centrosomal accumulation of Cdc25C is cell cycle-dependent. (A) Indirect immunofluorescence of HeLa cells expressing GFP-centrin with the C-20 antibody. Representative micrographs of cells with unduplicated (G_1 or early Sphase), duplicating (S phase) and duplicated & separated centrosomes (G_2) are shown. Bar: 5 μ m. Images were acquired with a 100X objective. (B) Quantification of Cdc25C levels at non duplicated (ND), duplicating or duplicated centrosomes. Standard error bars were calculated from quantification of approximately 100 cells from three independent experiments. (C) Centrosomal localisation of endogenous Cdc25C (A) correlates with the presence of Cyclin A (B); merge (C). Bar: 20 μ m. Images were acquired with a 40X objective. (D) Quantification of relative intensities of centrosomal Cdc25C and of Cyclin A in individual cells reveals a trend indicative of a correlation between accumulation of Cyclin A during S-phase and G_2 and centrosomal accumulation of Cdc25C. Each dot represents a cell.

bleached population being replaced with a half-recovery time of 2.3 \pm 1.5 s. Centrosomal EGFP-Cyclin B1 was equally dynamic with a half-recovery time of 2.51 \pm 1.4 s, as also observed by others (Richardson J and Pines J, personal communication).

Molecular basis for centrosomal localisation of Cdc25C. To gain insight into the molecular basis for centrosomal localisation of Cdc25C, we asked whether it might be mediated through specific sequence determinants, functional domains or protein partners. The N-terminal, regulatory domain of Cdc25 phosphatases harbours residues involved in substrate and partner recognition, including motifs involved in Cyclin B1 and 14-3-3 binding.⁴⁴⁻⁴⁷ Moreover, the catalytic domain of Cdc25B bears several "hot spot" residues, which are conserved in all three Cdc25 isoforms, as well as a C-terminal stretch of amino acids involved in recruitment of its Cdk substrate.⁴⁸⁻⁵⁰ We therefore generated a series of constructs expressing mRFP-tagged Cdc25C variants, deleted for its N-terminal domain (construct 286–473), or for its C-terminal domain with or without the consensus 14-3-3 binding site and adjacent NLS (constructs 1–285 and 1–180, respectively). A schematic representation of the different deletion variants and mutants of mRFP-Cdc25C generated for the purpose of this study is shown in Figure 4A. These constructs were microin-

jected into synchronized HeLa cells in G_2 and microinjected cells in which the different mRFP-Cdc25C variants localised to centrosomes were scored (Fig. 4B–D). We found that only the C-terminal domain localised to centrosomes with similar efficiency to full-length mRFP-Cdc25C, and preferentially in cells in G_2 . However, the longer N-terminal construct (1–285), but not the shorter one, partially relocalised to centrosomes in prophase, indicating that the region between residues 180 and 285 contributes to centrosomal localisation of Cdc25C in prophase. To further investigate whether catalytic activity, Cdk or 14-3-3 binding had any influence on centrosomal accumulation, we generated a catalytically inactive form of mRFP-Cdc25C (C377S), two substrate-binding "hotspot" mutants R392L and Y401A (corresponding to R488 and Y497 in Cdc25B),⁴⁸ and a form which no longer binds 14-3-3 (S216A). As shown in Figure 4E, none of these single amino acid substitutions impeded centrosomal localisation of Cdc25C.

Phosphatase-dead and substrate-trapping mutants of Cdc25C accumulate at centrosomes with phospho-Tyr15 Cdk1. Previous studies report that Cdc25C does not play any role in mitotic entry, in contrast to the Cdc25B isoform, which is responsible for triggering activation of MPF at the G_2 /M transition.^{12,13} However, we

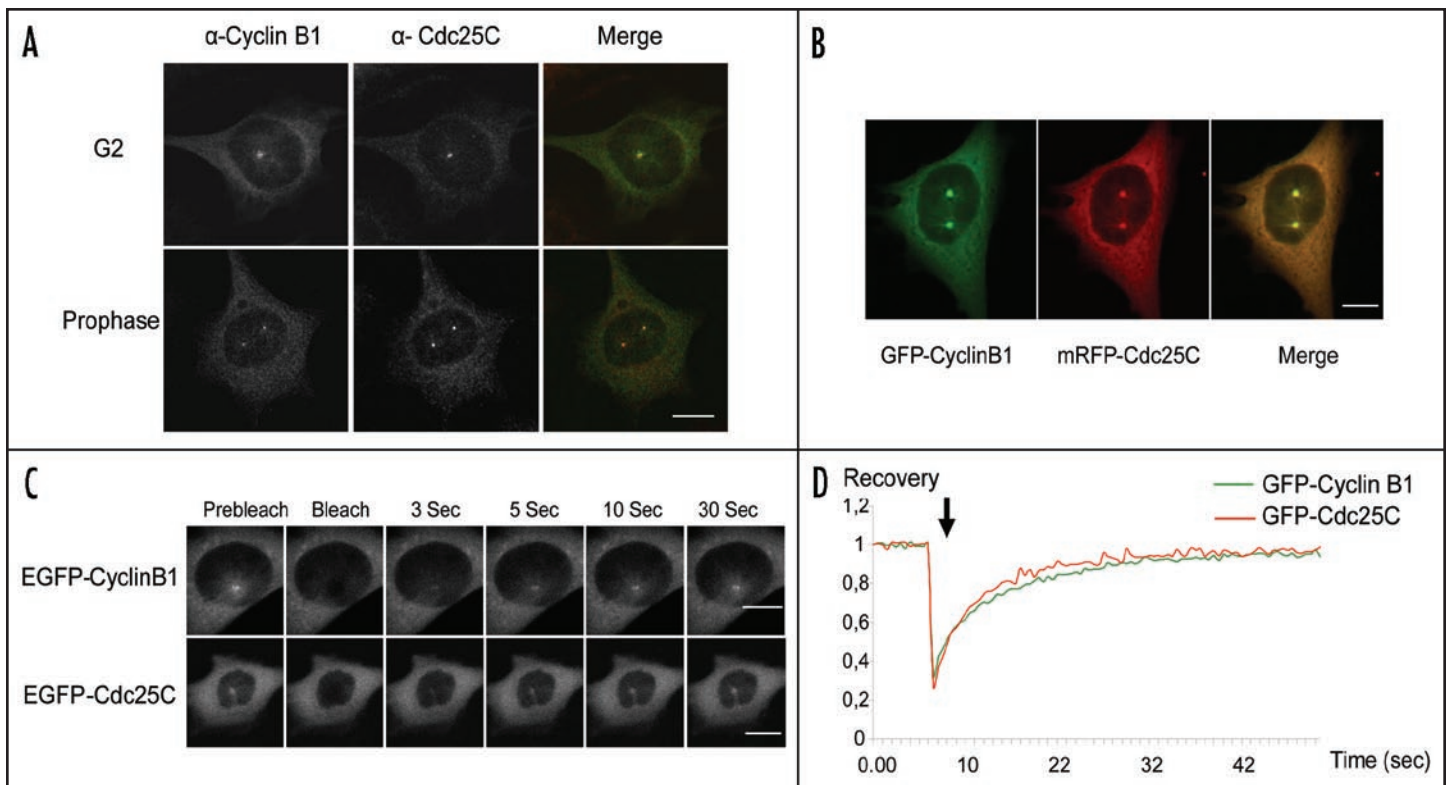


Figure 3. Cdc25C and Cyclin B1 co-localise at centrosomes in G_2 and prophase and exhibit high mobility and dynamic exchange with the cytoplasm. (A) Indirect immunofluorescence of endogenous Cdc25C and Cyclin B1 in G_2 (upper) and in prophase (lower) in HeLa cells using the C20 and GNS1 antibodies, respectively. Bar: 10 μ m. Images were acquired with a 40X objective. (B) Ectopically expressed mRFP-Cdc25C and EGFP-Cyclin B1 colocalise at centrosomes in prophase following microinjection of constructs into HeLa cells synchronized in G_2 . Bar: 5 μ m. Images were acquired with a 63X objective. (C) FRAP of centrosomal EGFP-Cdc25C and EGFP-Cyclin B1 in HeLa cells. Representative images of a typical FRAP experiment are shown for EGFP-Cyclin B1 (upper) and EGFP-Cdc25C (lower). Prebleach: cells prior to photobleaching; Bleach: at time t_0 when photobleaching pulse was performed; other time points throughout the course of the experiment are indicated above each panel. Bar: EGFP-Cyclin B1: 5 μ m. EGFP-Cdc25C: 10 μ m. (D) Typical recovery curves for EGFP-Cyclin B1 and EGFP-Cdc25C.

found that microinjection of a construct expressing mRFP-Cdc25C into synchronized HeLa cells in G_2 (and only in G_2), lead to a net induction of mitosis compared to mRFP (Fig. 5A and B), although much less significant than Cdc25B at this stage (data not shown). In contrast, the C377S, R392L and Y401A mutants were defective in their ability to promote entry into mitosis, whether or not coinjected with Cyclin B1, despite the presence of endogenous Cdc25C, therefore behaving as dominant negative mutants. Moreover, we found that these mutants accumulated at centrosomes and promoted accumulation of phospho-Tyr15 Cdk1 at centrosomes, before cells die (Fig. 5C), inferring that the defect in mitotic induction was related to a defect in Cdk1 activation, which is initiated at the centrosome. Closer examination of these cells revealed that several were still capable of separating their centrosomes before dying (Suppl. Fig. 2), indicating that initial Cdc25B-dependent activation of Cdk1-Cyclin B1 required for centrosomal separation⁵¹ was probably not affected.

Discussion

In mammalian cells, Cdc25C is sequestered in the cytoplasm during interphase by 14-3-3,³²⁻³⁵ and then translocates into the nucleus at the G_2 -M transition, following a series of phosphorylation and dephosphorylation events.³⁷⁻⁴² In this study we report that a fraction of Cdc25C localises to centrosomes in a cell cycle-dependent fashion, as of late S phase and throughout G_2 and

mitosis, inferring a biological function for Cdc25C at this organelle during these stages. Moreover, we find that Cdc25C and Cyclin B1 colocalise at centrosomes in G_2 and in prophase, and that they both undergo a dynamic exchange between the centrosome and the cytoplasm, suggesting a functional interaction between these two partners. It is widely accepted that Cdc25B triggers initial activation of Cdk1-Cyclin B1 at the centrosome,¹⁸ whereas Cdc25C has been thought to act subsequently to amplify MPF activity during mitosis in the nucleus.^{12,13,16,20} However, we find that catalytically inactive or substrate-binding "hotspot" mutants of Cdc25C promote centrosomal accumulation of phosphoTyr15-Cdk1 and impair entry into mitosis. Taken together these findings suggest that centrosomal localisation of Cdc25C is associated with substrate recognition and dephosphorylation, and infer that Cdc25C contributes to activation of Cdk1-Cyclin B1 at the G_2 /M transition. We propose that Cdc25C may participate in amplification of Cdk1-Cyclin B1 activity following initial activation by Cdc25B, and that this process is initiated at the centrosome, but then further propagated throughout the cytoplasm thanks to the dynamic behavior of both Cdc25C and Cyclin B1.

In addition to being the major microtubule-organizing center of mammalian cells, the centrosome acts as an integrative scaffold which facilitates interactions between mitotic players by increasing their local concentration. In fact, a growing number of reports document the recruitment and functional activation of several mitotic

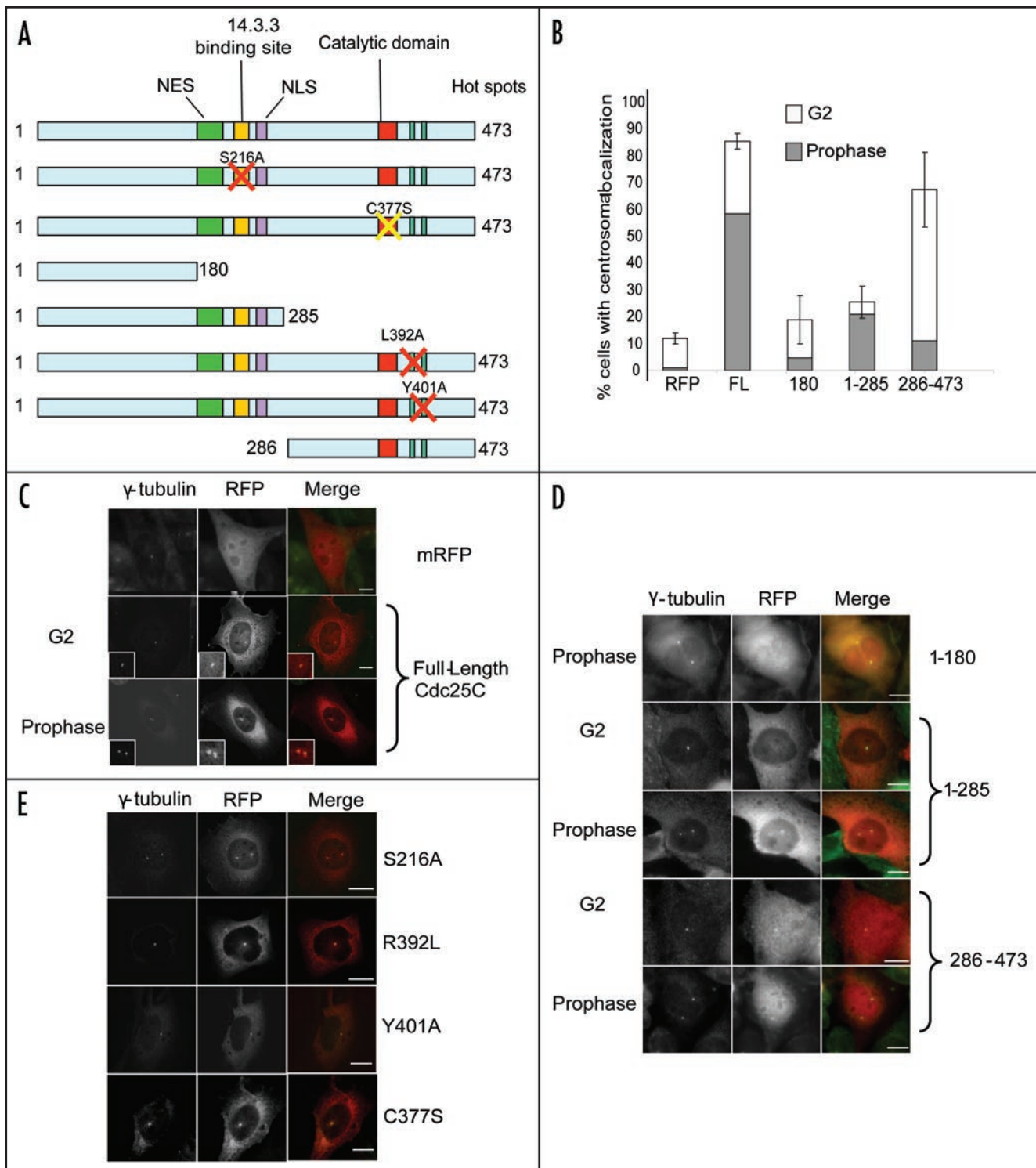


Figure 4. Molecular basis for centrosomal localisation of Cdc25C. (A) Schematic representation of the mRFP-Cdc25C mutants and truncation constructs generated: full-length wildtype (FL); S216A, C377S, R392L, Y401A, N-terminal domains 1–180 and 1–285; C-terminal domain 286-end. (B) Statistical analysis of the centrosomal localisation of the different mRFP-Cdc25C variants described in (A) following microinjection into HeLa cells in G₂. For each construct, approximately 120 cells were scored. Results are shown \pm SE bars ($n = 3$). (C) Typical micrographs of HeLa cells in G₂ and prophase illustrating the subcellular localisation of mRFP and wild-type Cdc25C, (D) of different mRFP-Cdc25C truncation constructs (E) and of single-site mutants: S216A, R392L, Y401A and C377S. Images were acquired with a 63X objective. Bar 10 μ m.

regulators at the centrosome, including Cdk1-Cyclin B1, Cdc25B, Polo-like kinases, Aurora kinases and Pin1. Initial appearance of active Cdk1-Cyclin B1 at centrosomes in prophase is thought to involve Plk1-mediated Cyclin B1 phosphorylation,¹⁸ and a recent

model for the interaction between Plk1 and Cdc25C suggests that their interaction is likely to occur at the centrosome.⁵² This report is perfectly in line with our findings and provides a functional rationale for the centrosomal localisation of Cdc25C.

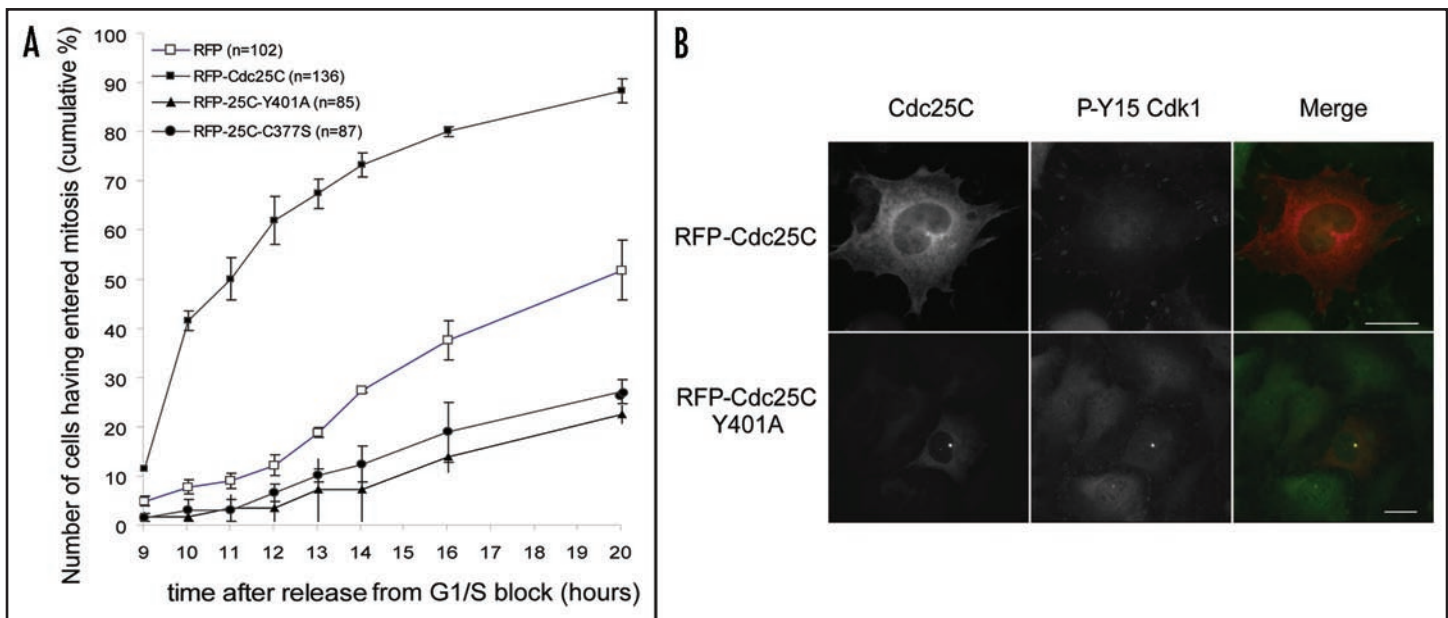


Figure 5. Phosphatase-dead and hotspot mutants of Cdc25C delay entry into mitosis by accumulating at the centrosome together with phospho-Y15 Cdk1. (A) Kinetics of mitotic entry monitored by cell rounding of synchronized HeLa cells microinjected 6 hours after release from hydroxyurea with plasmids encoding mRFP, mRFP-Cdc25C, phosphatase-dead mRFP-C377S and hotspot mutant mRFP-Y401A. Results are shown as a mean \pm SE bars ($n = 3$). (B) Micrographs of HeLa cells illustrating the subcellular localisation of phospho-Y15 Cdk1 in cells microinjected with wild type mRFP-Cdc25C (upper) or the Y401A mutant (lower). Bar: 10 μ m. Images in (B and C) were acquired with a 20X objective, images in (D) with a 40X objective.

Our attempts to characterise the molecular basis for centrosomal localisation of Cdc25C reveal that it is primarily dependent on its C-terminal domain, in line with its role in substrate recognition, and altogether independent of the first 180 N-terminal residues. This region is involved in interactions with numerous partners, including cyclins^{45,46} and Plk1.⁵² Therefore our findings infer that centrosomal targeting of Cdc25C is not mediated through Plk1 or cyclin binding, and that interactions between these partners at the centrosome itself occur following their recruitment to this organelle. In contrast, the region between residues 180 and 285 clearly participates in recruitment of Cdc25C to centrosomes in prophase. Interestingly this region harbours the NES and the NLS, a Cyclin B1 binding site, the 14-3-3 binding site, which bears the S216 phosphorylation site, and the adjacent S214 mitotic phosphorylation site. In particular, phospho-S216-Cdc25C is dephosphorylated upon 14-3-3 dissociation at the G₂/M transition, and this is followed by activating phosphorylation of Cdc25C on several residues, including S214, which prevents rebinding of 14-3-3.^{39,40,53} In this study, we have shown that centrosomal localisation of Cdc25C is independent of 14-3-3 binding. As such, it seems reasonable to surmise that phosphorylation of S214 may further contribute to localization of Cdc25C at the centrosome in prophase, although further investigations will be required to address this issue.

In conclusion, our study provides insight into the molecular features and dynamics of the centrosomal localisation of Cdc25C, thereby suggesting an unexpected function for this phosphatase at the G₂/M transition. In an integrated model, we propose that complete activation of Cdk1-Cyclin B1 required for robust and timely entry into mitosis is the result of the cooperation between Cdc25B and Cdc25C, the former triggering initial activation of centrosomal Cdk1-Cyclin B1, the latter ensuring amplification and

propagation of kinase activity. Issues that remain to be addressed include understanding why a fraction of Cdc25C remains localised to centrosomes once entry into mitosis is triggered, nuclear envelope breakdown, and spindle assembly have proceeded, and identifying what triggers initial association of Cdc25C to the centrosome, and what primes its dissociation at the end of mitosis.

Materials and Methods

Cell culture, synchronisation, transfection and microinjection. HeLa cells were cultured in DMEM supplemented with 10% FCS, 1 mM penicillin and 1 mM streptomycin at 37°C in an atmosphere containing 5% CO₂. NHF cells were graciously provided by J. Piette (CNRS, Montpellier, France). HeLa cells stably expressing GFP-Centrin were a kind gift from M. Bornens (Institut Curie, Paris, France) and were cultured with 0.5 mg/ml G418 (Calbiochem). siRNA transfections were performed twice at 24 h interval at a 50 nM concentration with OligofectamineTM (Invitrogen) and cells collected after 72 h. HeLa cells were synchronized with 1 mM hydroxyurea (Sigma) and released for 6 h to reach G₂. Microinjections were performed with a semi-automatic Eppendorf microinjection apparatus.

Antibodies, western blotting and indirect immunofluorescence. Antibodies against Cyclin B1 (GNS1, sc-245) and Cdc25C (C-20, sc-327) were purchased from Tebu-Bio (Santa-Cruz), anti-Cdc25A (CS-3656), anti-Cdc25B (CS-9525) and anti-pTyr15-Cdk1 were from Cell Signalling Technology. These primary antibodies were used at 1:50 dilution for indirect immunofluorescence, 1:500 for Western blotting. A 40 fold excess (2 μ M) of immunization peptide (Santa Cruz, sc-6392 P) was used to titrate the C-20 antibody. Anti-gamma tubulin (GTU-488, Sigma), was used at a 1:1000 and 1:10000 dilution for Western blotting and for indirect immunofluorescence, respectively. Monoclonal anti-CTR453 antibody raised against

AKAP-450 was kindly provided by M. Bornens (Curie Institute, France) and used at a 1:10 dilution for indirect immunofluorescence. Horseradish peroxidase-coupled secondary antibodies for Western blotting were purchased from GE Healthcare and Alexa488 or Alexa594-coupled secondary antibodies for indirect immunofluorescence were from Molecular Probes.

For Western blotting, cell extracts were prepared in lysis buffer containing 50 mM TrisHCl, pH 7.4, 150 mM NaCl, 0.1% NP40, 0.1% Deoxycholate, 2 mM EDTA, 1 mM PMSF, Complete™ protease inhibitors (Roche), 50 mM NaF, 40 mM β -Glycerophosphate, 1 mM Na_3VO_4 and dosed in a BCA assay (Pierce).

For indirect immunofluorescence, cells were plated onto glass coverslips coated with fibronectin (Sigma). Cells were fixed with PFA 4% (Electron Microscopy Science) for 10 min and extracted with methanol for 5 minutes at -20°C . All incubations and washes were performed with PBS/BSA 1%, 0.1% Tween 20. Nuclei were stained with Hoechst 33342 (Sigma), and coverslips were mounted in Prolong Antifade Reagent (Invitrogen).

Microscopy. Epifluorescence images were acquired on a ZEISS AxioImager Z1 microscope with a Plan Apo 20x objective/0.8 NA (numerical aperture), an EC Plan Neofluar 40x objective/1.3 NA, a PlanApo 63x objective/1.4 NA or an EC Plan Neofluar 100x objective/1.3 NA, equipped with a Coolsnap HQ camera (Photometrics) and piloted by the Metamorph software (Universal Imaging). Confocal images and FRAP experiments were performed with a ZEISS LSM 510 confocal microscope. EGFP was excited with the 488-nm line of the Argon laser. After 20 prebleach scans (2 scans/s), a region of interest (ROI) was bleached with 25 iterations at 100% laser power, and fluorescence recovery was sampled for 2 min every 500 milliseconds. For time-lapse microscopy, cells were observed with a Zeiss Axiovert microscope with an incubation chamber containing 5% CO_2 and thermostated at 37°C , equipped with a Zeiss Plan Neofluar objective 10x/0.3 NA or with a Zeiss LD A-Plan objective 20x/0.8 NA. Fluorescence intensity quantifications were performed essentially as described by Lindqvist et al., (2007) on 10 image stacks with a 1 μm spacing, using the Metamorph software (Universal Imaging). Regions of interest (ROI) were selected in the nucleus, in the cytoplasm and outside the cell to obtain background values. For centrosomes, maximal intensity was measured and cytoplasmic intensity subtracted to correct protein level variations.

Centrosome purification. Centrosomal fractions were purified on a sucrose gradient from HeLa cells synchronised in G_2 , according to the original protocol developed by Moudjou and Bornens.⁵⁴

Molecular biology. cDNA encoding human Cdc25C was cloned into pcDNA3.1-mRFP1 (kindly provided by T.Ng, King's College London, UK), and all mutants were generated according to standard procedures. Plasmids expressing EGFP-Cyclin B1 and EGFP-Cdc25C were generously provided by D. Chang (Hong Kong University of Science and Technology, Hong Kong, China) and P.J. Stambrook (University of Cincinnati College of Medicine, Cincinnati, USA), respectively. Smart-Pool™ siRNAs targeting Cdc25C and control siRNA pools were obtained from Dharmacon.

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Note

Supplementary materials can be found at:

www.landesbioscience.com/supplement/BonnetCC7-13-Sup.pdf

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