

The Localization of Human Cyclins B1 and B2 Determines CDK1 Substrate Specificity and Neither Enzyme Requires MEK to Disassemble the Golgi Apparatus

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Abstract. In this paper, we show that substrate specificity is primarily conferred on human mitotic cyclin-dependent kinases (CDKs) by their subcellular localization. The difference in localization of the B-type cyclin-CDKs underlies the ability of cyclin B1-CDK1 to cause chromosome condensation, reorganization of the microtubules, and disassembly of the nuclear lamina and of the Golgi apparatus, while it restricts cyclin B2-CDK1 to disassembly of the Golgi apparatus. We identify the region of cyclin B2 responsible for its localization and show that this will direct cyclin B1 to the Golgi apparatus and

confer upon it the more limited properties of cyclin B2. Equally, directing cyclin B2 to the cytoplasm with the NH₂ terminus of cyclin B1 confers the broader properties of cyclin B1. Furthermore, we show that the disassembly of the Golgi apparatus initiated by either mitotic cyclin-CDK complex does not require mitogen-activated protein kinase kinase (MEK) activity.

Key words: cyclin • CDK • mitosis • protein kinase • Golgi apparatus

Introduction

Cyclins play a vital role in controlling progress through the eukaryotic cell cycle. They are required at each cell cycle transition to activate their partner cyclin-dependent kinase (CDK),¹ for commitment to proliferation (at START or the restriction point), initiation of DNA replication, and initiation of mitosis (for reviews see Pines, 1995; Morgan, 1997). Different cyclin-CDK complexes are required at each of these steps. In budding and fission yeasts, only one CDK is required for the cell cycle and this binds different cyclins at specific stages in the cell cycle. In animal cells, different CDKs are active at different stages of the cell cycle, but again each is able to bind more than one cyclin.

The question of whether and how cyclins impart specificity to their partner CDK is the subject of some debate (Cross et al., 1999; Geng et al., 1999; for review see Roberts, 1999; Cross and Jacobson, 2000; Jacobson et al., 2000). In fission yeast, a single B-type cyclin-CDK complex, *cdc13-cdc2*, is able to initiate either DNA replication (S phase)

or mitosis (M phase) depending on the amount of kinase activity. Low kinase levels promote S phase, whereas high amounts promote mitosis (Fisher and Nurse, 1996). These results indicate that a cyclin may not change the substrate specificity of a CDK because its biological action could be determined by its relative affinity for S phase compared with M phase substrates (Stern and Nurse, 1996). In contrast, there are clear differences in the biological properties of different cyclin-CDK complexes in budding yeast. Budding yeast have two subfamilies of cyclins that cannot substitute for each other: (a) three CLN cyclins that act at START; and (b) six different B-type cyclins (CLB) that control progress through S and M phases. There is also specificity among the CLBs because the S phase CLB5 and the M phase CLB2 cyclins cannot substitute for each other when expressed at physiological levels (Cross et al., 1999; Jacobson et al., 2000).

The molecular basis for the functional difference between CLB2 and CLB5 has not been definitively defined, but attention has focused on differences in the “hydrophobic patch” that appears to play an important role in substrate recognition by the cyclin A-CDK2 complex in human cells (Schulman et al., 1998). The hydrophobic patch is on the outer face of helix 1 of the cyclin box (Jeffrey et al., 1995; Noble et al., 1997). Evidence from crystal structures (Russo et al., 1996) and mutation studies impli-

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¹Abbreviations used in this paper: CDK, cyclin-dependent kinase; ERK, extracellular signal-regulated kinase; GFP, green fluorescent protein; LMB, leptomycin B; MAP, mitogen-activated protein; MEK, MAP kinase kinase; NAGT, N-acetylglucosaminyltransferase 1.

cated the hydrophobic patch in binding to the “Cy motif” (R × L) of substrates such as p107 (Zhu et al., 1995), CDK inhibitors such as p21^{Cip1/Waf1} and p27^{Kip1} (Chen et al., 1995; Russo et al., 1996), and the CDK activator Cdc25A (Saha et al., 1997). Thus, the hydrophobic patch may act as a substrate targeting and specificity domain. In indirect support of this, a form of CLB5 carrying a mutation in the hydrophobic patch was partially defective in several assays, including the activation of late-firing origins of DNA replication in S phase cells (Cross et al., 1999). Similarly, mutating the hydrophobic patch in CLB2 reduced its ability to rescue yeast with a temperature-sensitive Clb2 gene against the background of a strain lacking Clb1, Clb3, and Clb4 (Cross and Jacobson, 2000).

There are two mammalian B-type cyclins, cyclins B1 and B2, that differ in their NH₂ termini but are 57% identical in the rest of the protein (Chapman and Wolgemuth, 1993; Jackman et al., 1995; Brandeis et al., 1998). Both cyclins bind to CDK1 and are active only in mitosis (Jackman et al., 1995), but only cyclin B1 is essential. Mice lacking cyclin B1 die in utero. Mice lacking cyclin B2 are smaller than normal and have reduced litter sizes, indicating that cyclin B2 does confer a growth advantage (Brandeis et al., 1998). What underlies this difference between the cyclins? Cyclin B2 is expressed at a much lower level than cyclin B1; therefore, it is possible that cyclin B2 cannot compensate for cyclin B1 because there is not enough of the protein in the cell (Brandeis et al., 1998). However, there is also a striking difference between the proteins in their subcellular localizations. Cyclin B1 is primarily cytoplasmic but constantly shuttles between the nucleus and the cytoplasm in interphase (Hagting et al., 1998; Toyoshima et al., 1998; Yang et al., 1998). At the end of prophase, it rapidly translocates into the nucleus (Furuno et al., 1999; Hagting et al., 1999) and then binds to the mitotic apparatus (Pines and Hunter, 1991; Hagting et al., 1998; Clute and Pines, 1999). In contrast, mammalian cyclin B2 is primarily bound to the Golgi apparatus in both interphase and mitosis (Jackman et al., 1995; Brandeis et al., 1998).

In mammalian cells, the Golgi apparatus disassembles at mitosis in a carefully orchestrated manner (Misteli and Warren, 1995a), such that approximately equal numbers of vesicles are partitioned to each daughter cell (Shima et al., 1997). This process is regulated by phosphorylation (Misteli and Warren, 1995a,b; Lowe et al., 1998), but there is a continuing debate over which protein kinase(s) is responsible. There is disagreement over whether the kinase is a cyclin-CDK (Lowe et al., 1998, 2000), mitogen-activated protein (MAP) kinase kinase (MEK) (Acharya et al., 1998; Colanzi et al., 2000), or both (Kano et al., 2000; for review see Nelson, 2000).

In this paper, we have asked whether the subcellular distribution of the cyclins specifies the biological activity of the cyclin-CDK complex and whether they can disassemble the Golgi apparatus without MEK. We find that CDK1 has a much more limited effect on subcellular architecture when it is activated by the Golgi-associated cyclin B2 than when it is activated by cyclin B1. Moreover, when cyclin B1 is targeted to the Golgi apparatus by the NH₂ terminus of cyclin B2, it takes on the more restricted properties of cyclin B2. These results may provide an explanation for why cyclin B2 is unable to complement cyclin B1 and demonstrate the

profound influence of subcellular localization on the biological role of a cyclin-CDK. Moreover, we show that in living cells, either cyclin B1-CDK1 or cyclin B2-CDK1 is sufficient to initiate Golgi breakdown and that MEK is not required for Golgi apparatus disassembly in mitosis.

Materials and Methods

Cell Culture and Synchronization

HeLa and CHO cells were cultured in DME and Hams F-12 media, respectively, supplemented with 10% FCS at 37°C, 10% CO₂. To obtain G0/G1 phase cells, CHO cells were cultured in Hams F-12 plus 0.1% FBS on glass coverslips for at least 3 d and then microinjected in the presence of 2% serum. To inhibit MEK activity, serum-starved cells were incubated in 20 μM PD98059 and 25 μM U0126 for 1 h before injection and subsequently for the duration of the experiment. Fresh PD98059 was added every hour because of its short half-life in solution. To analyze entry into mitosis in the presence of MEK inhibitors, CHO cells were arrested in G0 by serum starvation for 36 h and then released by addition of serum. After 18 h (G2 phase), 20 μM PD98059 and 25 μM U0126 were added. Fresh medium and inhibitors were added every hour until cells had reentered G1 phase ~6 h later. HeLa cells were synchronized using a thymidine and aphidicolin regime as described previously (Heintz et al., 1983).

Construction of Chimeras and Expression Constructs

Chimeras were constructed by overlapping PCR using VENT polymerase (Biolabs). The sequences of the oligonucleotides used are available on request. Constructs were cloned into eukaryotic expression vectors and sequenced on an automatic sequencer. The pCMX-cyclin B1-MmGFP, pCMX-cyclin B1, and pCMX-cyclin B2 constructs have been described previously (Jackman et al., 1995; Hagting et al., 1998). The cyclin B1-B2 chimera was constructed by ligating the myc epitope-tagged NH₂ terminus of cyclin B1 (base pairs 1-431) to the cyclin box and second cyclin fold of cyclin B2 (base pairs 318-1191) via a Hind III restriction enzyme site. The cyclin B2-B1 chimera was constructed by ligating the myc epitope-tagged NH₂ terminus of cyclin B2 (base pairs 1-391) to the cyclin box and second cyclin fold of cyclin B1 (base pairs 503-1302) using Pst I and Nsi I restriction enzyme sites.

Microinjection, Transfection, and Immunofluorescence

Cells were injected in the nucleus with expression constructs as described previously (Hagting et al., 1998). Cells were incubated in CO₂-independent medium without phenol red (GIBCO BRL) during injection and subsequently incubated for 3-6 h at 37°C, 10% CO₂ in Hams F-12 medium plus 2% FCS. For transfection experiments, 293T cells (2 × 10⁷) were transfected using calcium phosphate precipitation with 20 μg of plasmids encoding cyclin B1 or B2 or the chimeras and 20 μg of CDK1^{AF} expression vector with 30 μg of pING plasmid DNA for 24 h. Cells were harvested 12 h after transfection. For immunofluorescence analysis, cells were fixed in ice-cold methanol for 2 min. Antibodies were diluted in 3% BSA and 0.2% Tween containing PBS. When using the phospho-specific antibody, 2 μM microcystin was included in the antibody dilution buffer. Primary antibodies were used at the following dilutions and incubated for 1 h: anti-human cyclin B1 rabbit polyclonal (1:400), anti-mouse cyclin monoclonal B1 V143 that does not cross-react with human cyclin B1 (1:200) (a gift from J. Gannon and Tim Hunt, Imperial Cancer Research Fund, London, UK), anti-cyclin B2 rabbit polyclonal (1:25), anti-mannosidase II rabbit polyclonal (1:50), anti-giantin monoclonal antibody (1:100), anti-β-tubulin mouse monoclonal (1:100) (Amersham Pharmacia Biotech), anti-nuclear lamin rabbit polyclonal (1:200) (a gift from Larry Gerace, The Scripps Research Institute, La Jolla, CA). Secondary antibodies linked to FITC, Texas red, or Cy-5 (Amersham Pharmacia Biotech) were used at 1:200 dilution and incubated for 30 min. Antibody incubations were followed by three washes of PBS containing 0.2% Tween. DNA was stained using TOTO-3 dye (Molecular Probes) in the mounting media. Cells were viewed on a Nikon Eclipse microscope equipped with an MRC 1024 confocal laser scanning microscope system using a 60× 1.4 NA oil immersion lens.

Immunoblotting and Immunoprecipitation

Cells (5×10^6) were lysed in NP-40 lysis buffer (100 mM Tris, pH 8, 300 mM NaCl, 2 mM EDTA, 2 mM EGTA, pH 8, 2% NP-40, 2 mM PMSF, 20 mM NaF, 2 mM Na_2VO_4 , 2 $\mu\text{g/ml}$ leupeptin, 2 $\mu\text{g/ml}$ aprotinin) for 20 min on ice. Microcystin (2 μM) was included when analyzing samples for MEK activity. For immunoblotting to detect MAP kinase, 50–80 μg of protein from the lysate was diluted in 5 \times reducing sample buffer. Samples were resolved by SDS-PAGE and transferred to PVDF membrane (Immobilon). The membrane was blocked for 60 min in 5% milk PBS and incubated with 1 $\mu\text{g/ml}$ of anti-myc tag epitope mAb (9E10) or 0.5 $\mu\text{g/ml}$ of the anti-HA tag epitope mAb (12CA5). Anti-phospho-p42/p44 MAP kinase antibody (Cell Signaling Technology) and anti-extracellular signal-regulated kinase (ERK)1 and ERK2 antibodies (Promega) were used at 1:1,000. Secondary antibodies, HRP-conjugated goat anti-mouse IgG or goat anti-rabbit polyclonal antibody (Zymed Laboratories), were detected using ECL (Amersham Pharmacia Biotech). For immunoblotting to detect cyclin B1 levels in microinjected cells, G0 CHO cells were seeded on CELLocate coverslips (Eppendorf) and microinjected with plasmids encoding cyclin B1, NAGT-GFP, and CDK1^{AF} and incubated for 6 h. NAGT-GFP positive cells were counted, and cells were lysed by spotting 5 μl of reducing sample buffer onto the center of the coverslip. After collecting the lysate, a further 5 μl of sample buffer was spotted onto the center of the coverslip, and the two samples were combined for analysis by one-dimensional SDS-PAGE. To collect known numbers of mitotic cells, cells were collected by mitotic shake-off without nocodazole treatment and diluted to a known concentration using a cell-counting chamber. Anti-cyclin B1 monoclonal V152 (a gift from J. Gannon and T. Hunt) that cross-reacts with human and rodent cyclin B1 was used at a final concentration of 2 $\mu\text{g/ml}$. For immunoprecipitation, cells were lysed in NP-40 lysis buffer, and the lysate was precleared with protein A/protein G (50:50) Sepharose beads. The supernatant was incubated for 2 h with an anti-myc rabbit polyclonal antibody (Upstate Biotechnology) (5 $\mu\text{g/ml}$ of lysate) followed by a 45-min incubation with protein A/protein G (50:50) Sepharose beads. Beads were washed three times in NP-40 lysis buffer and used either for kinase assays or resolved by SDS-PAGE and immunoblotted.

Histone H1 Kinase Assays

Immunoprecipitated cyclin-CDK complexes were washed three times in lysis buffer and once using kinase buffer (150 mM NaCl, 30 mM MgCl_2 , 60 μM ATP, 3 mM DTT, 3 mM Na_2VO_4 , 15 mM NaF). For each kinase reaction, 15 μl of washed beads was mixed with 15 μl of kinase buffer, 2.5 μCi of [γ -³³P]ATP, and 1.5 μg of histone H1 and incubated for 30 min at 30°C. The reaction was stopped by adding 5 μl of 4 \times SDS sample buffer. Histone kinase activity was assayed after one-dimensional SDS-PAGE using a PhosphorImager (Molecular Dynamics).

Results

G0/G1 Phase Cells Can Be Used to Analyze the Effects of Specific Mitotic CDKs

To elucidate any differences in the biological properties of mammalian B-type cyclins, we sought to analyze their effects in cells lacking any other mitotic cyclins. Therefore, we chose to express B-type cyclins in cells just after release from serum starvation (G0/G1) when they lack endogenous mitotic cyclins (Brandeis and Hunt, 1996). We used CHO cells because they have a well-defined Golgi apparatus, and we confirmed that serum-starved CHO cells do not have endogenous mitotic cyclins by both immunoblotting and immunofluorescence for cyclins A and B1 (Fig. 1, A and C). To generate active cyclin B-CDK1 complexes, we coexpressed cyclin B1 or B2 with a mutant form of CDK1, CDK1^{T14A,Y15F} (hereafter referred to as CDK1^{AF}) that cannot be inactivated by either the Wee1 or Myt1 kinases (Morgan, 1995). We coexpressed the proteins by one of two methods. In some experiments, we transfected the cDNAs under tetracy-

cline-inducible promoters into a CHO cell line carrying a tetracycline repressor. Immunoblotting extracts of the cells showed that both cyclin B1 and B2 were expressed to similar levels (Fig. 1 A) with similar kinetics and that their expression paralleled an increase in histone H1 kinase activity (data not shown). In other experiments, we analyzed cells by time-lapse microscopy and immunofluorescence after microinjecting expression constructs, encoding the proteins under the control of the CMV promoter. We could detect the proteins by confocal immunofluorescence microscopy 3 h after microinjection. By immunoblotting these cells and quantifying the signals with NIH Image, we found that microinjected cells reproducibly expressed approximately the same amount of cyclin B1 as an equivalent number of mitotic cells (Fig. 1 B). (Note that this analysis underestimated the amount of cyclin B1 in the mitotic samples because they were collected by mitotic shake-off without nocodazole treatment, and therefore contained some anaphase and telophase cells without any B-type cyclins.)

Theoretically, expressing active cyclin B-CDK1 complexes in G1 phase cells might have caused the levels of endogenous mitotic cyclins to increase if the kinases inactivated cyclin proteolysis by phosphorylating Cdh1. However, this would be unlikely to increase mitotic cyclin levels significantly because G0 cells had been shown to downregulate mitotic cyclin transcription (Piaggio et al., 1995; Zwicker et al., 1995). Nevertheless, we tested this possibility by staining cells for endogenous cyclins A and B1. This showed that none of the cells expressing ectopic cyclin B1- or B2- CDK1^{T14A,Y15F} complexes had any endogenous cyclin A or B1 (Fig. 1 C) throughout the time course of the experiments. Therefore, we could conclude that the effects we observed were due to the ectopic cyclins that we introduced.

Immunofluorescence analyses showed that the human B-type cyclins localized correctly in CHO cells. Cyclin B1 was cytoplasmic and partially associated with microtubules, whereas cyclin B2 localized to the Golgi apparatus (Fig. 1 D). We and others had shown that cyclin B1 continuously shuttled between the cytoplasm and the nucleus because of a nuclear export signal (NES) that counteracted its constitutive nuclear import. Human cyclin B1 also shuttled in G0/G1 phase CHO cells because both a myc epitope-tagged cyclin B1 (Fig. 1 E) and a cyclin B1-GFP fusion protein (not shown) relocalized to the nucleus when cells were treated with leptomycin B (LMB) to inhibit nuclear export (Fig. 1 E, compare a and c). We had also shown that the NH₂ terminus of cyclin B2 had a functional NES (Hagting et al., 1998) but found that the full-length protein did not enter the nucleus in G2 phase HeLa (not shown) or CHO cells treated with LMB (Fig. 1 E, compare b and d). Thus, cyclin B2 differed from cyclin B1 in that it remained continuously cytoplasmic, associated with the Golgi apparatus. This result agreed with our previous observations that cyclin B2 did not translocate to the nucleus at prophase and remained associated with membrane vesicles until it was degraded in mitosis (Jackman et al., 1995). From these results, we were able to conclude that human cyclins B1 and B2 had similar subcellular targeting activities in CHO cells as they did in human cells.

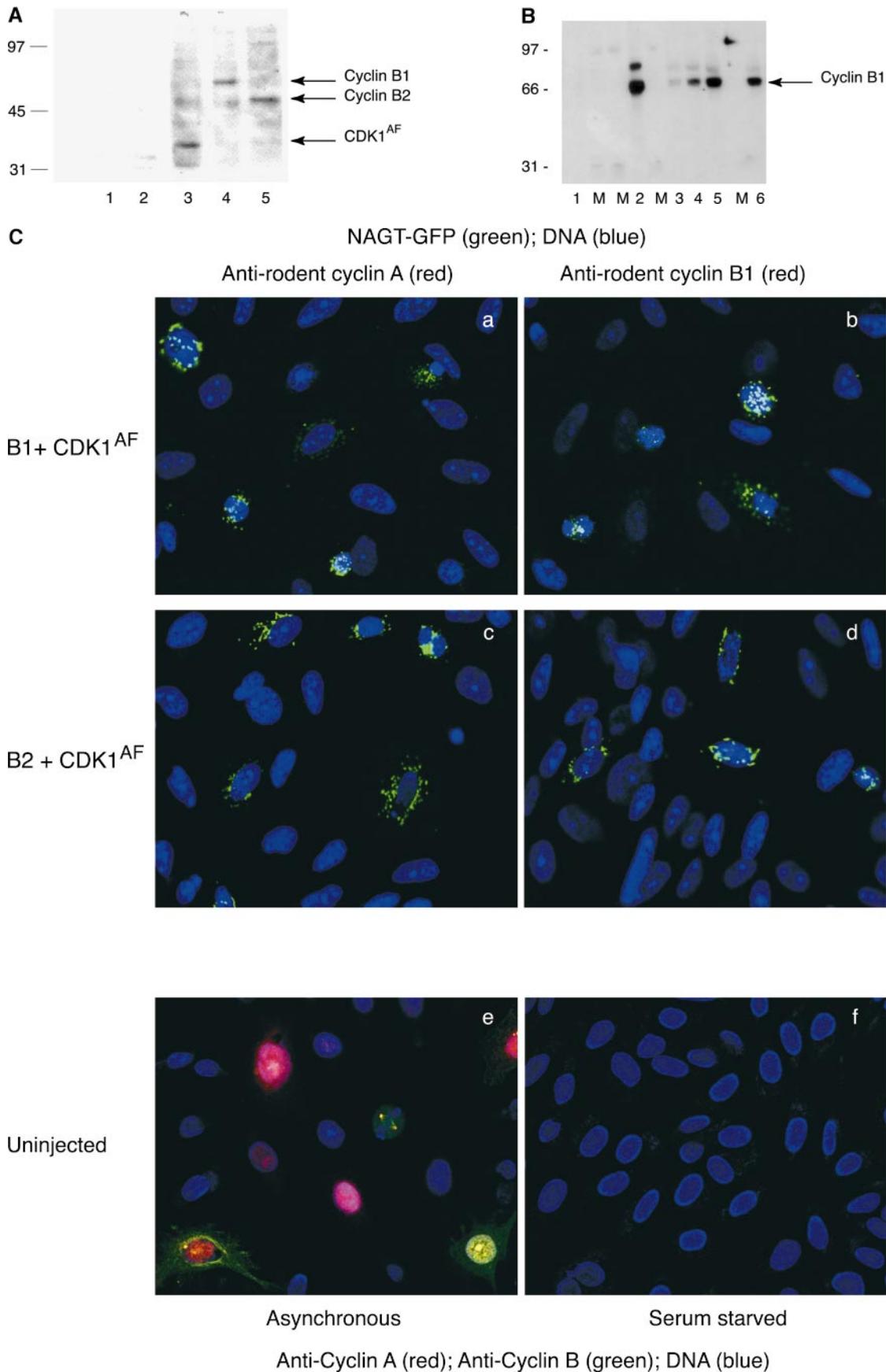
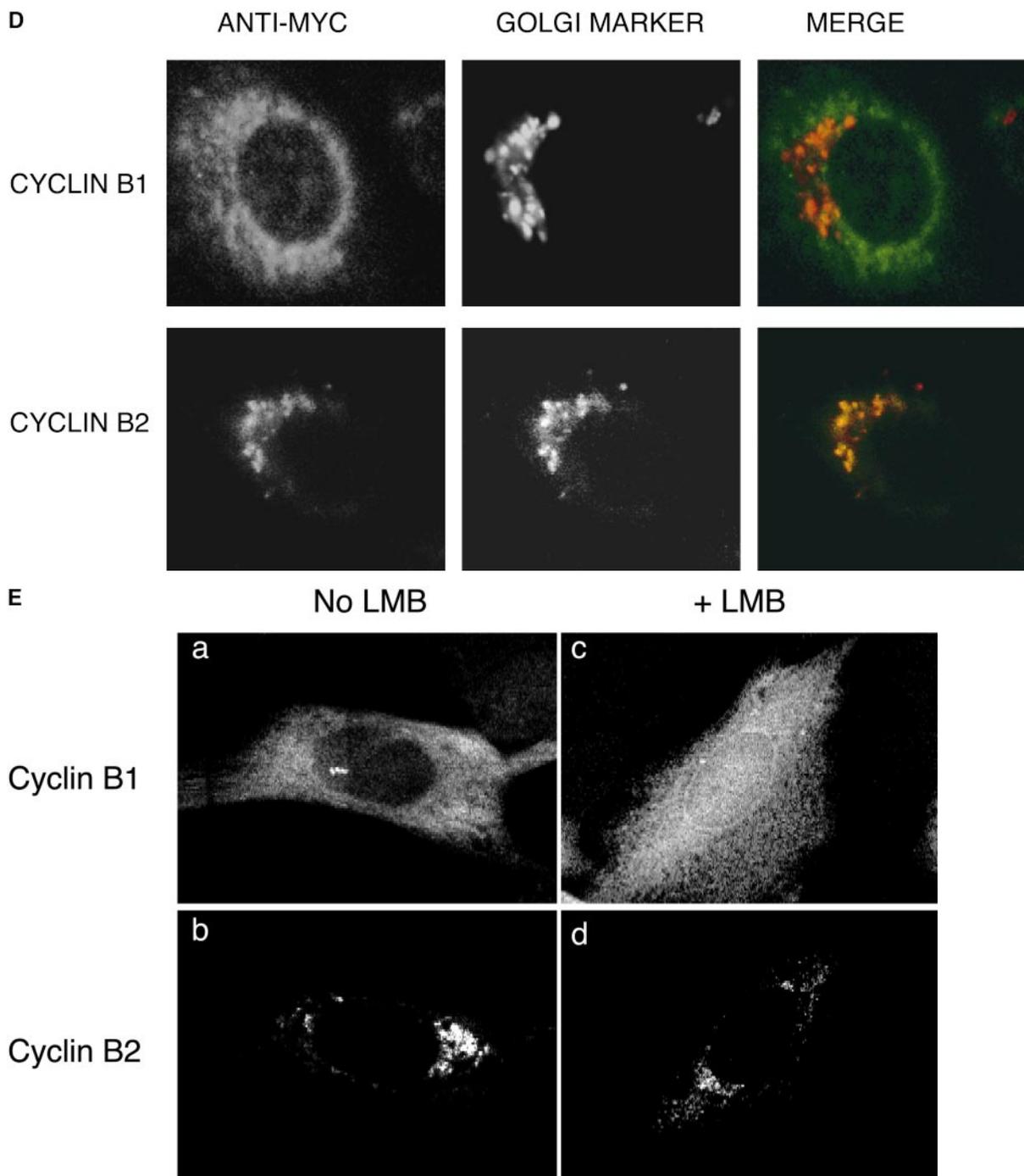


Figure 1. (A) Human B-type cyclins are expressed to similar levels in CHO cells. Serum-starved CHO cells were transfected with plasmids encoding human cyclin B1 or B2 and/or CDK1^{AF} all tagged with one copy of the myc epitope and under the “tetracyclin-OFF” promoter. Cells were incubated in the presence or absence of tetracyclin to repress or induce protein expression, respectively. 16 h after induction, cells were lysed, and the extracts were run on one-dimensional SDS-PAGE and then immunoblotted with the 9E10 mAb to detect the proteins. Lane 1, mock-transfected cells; lanes 2–5, transfected cells: (lane 2) cyclin B1 plasmid plus tetracyclin; (lane 3) Cdk1^{AF}; (lane 4) cyclin B1 minus tetracyclin for 16 h; (lane 5) cyclin B2 minus tetracyclin for 16 h. (B) Ectopic and endogenous cyclin



B1 are expressed at similar levels in CHO cells. Approximately 980 serum-starved CHO cells were microinjected with plasmids encoding myc epitope-tagged human cyclin B1 with CDK1^{AF}. 6 h after microinjection, cells were lysed and the samples were run on one-dimensional SDS-PAGE next to lysates from 250, 500, and 1,000 mitotic cells. Proteins were immunoblotted with an anticyclin B1 monoclonal antibody V152 that recognizes both human and rodent cyclin B1 to detect the proteins (a gift from J. Gannon and T. Hunt). Lane 1, uninjected cells; lane 2, cells injected with cyclin B1-CDK1^{AF}; lanes 3-5, 250, 500, and 1,000 mitotic HeLa cells; lane 6, 1,000 mitotic CHO cells. M, molecular mass marker lane. Results shown are representative of three independent experiments. (C) There are no detectable endogenous mitotic cyclins in serum-starved CHO cells with or without ectopic human cyclin-CDKs. (a-d) CHO cells were serum starved for 24 h and then microinjected with NAGT-GFP as a Golgi apparatus and injection marker (green) and TOTO-3 to visualize the DNA (blue) together with CDK1^{AF} and either cyclin B1 (a and b) or cyclin B2 (c and d). 6 h after microinjection, cells were fixed and stained with an anti-mouse cyclin A antibody (red) (a and c) or with an anti-rodent cyclin B1 monoclonal antibody V143 (red) that recognizes rodent B-type cyclins but does not cross-react with human B-type cyclins (a gift from J. Gannon and T. Hunt) (b and d). (e and f) Uninjected asynchronous (e) and serum-starved (f) CHO cells were costained with anticyclin A (red), anticyclin B1 (green), and TOTO-3 to visualize the DNA (blue). Results shown are representative of two independent experiments. (D and E) Human B-type cyclins localize correctly in CHO cells. Human cyclin B1 or B2 was tagged with one copy of the myc epitope and microinjected as cDNA under the CMV promoter into serum-starved CHO cells. 3 h after microinjection, cells were treated or not with LMB. Cells were stained with the 9E10 mAb to detect the cyclins. (D) Cells were costained with an antimannosidase II antibody to detect the Golgi apparatus (red in the merged images) and cyclin B1 (top row) or cyclin B2 (bottom row) (green in the merged images). (E) Cells expressing human cyclin B1 or B2 were fixed before (a and b) or after (c and d) treatment with 20 nM LMB for 45 min and stained for ectopically expressed cyclin B1 (a and c) or cyclin B2 (b and d).

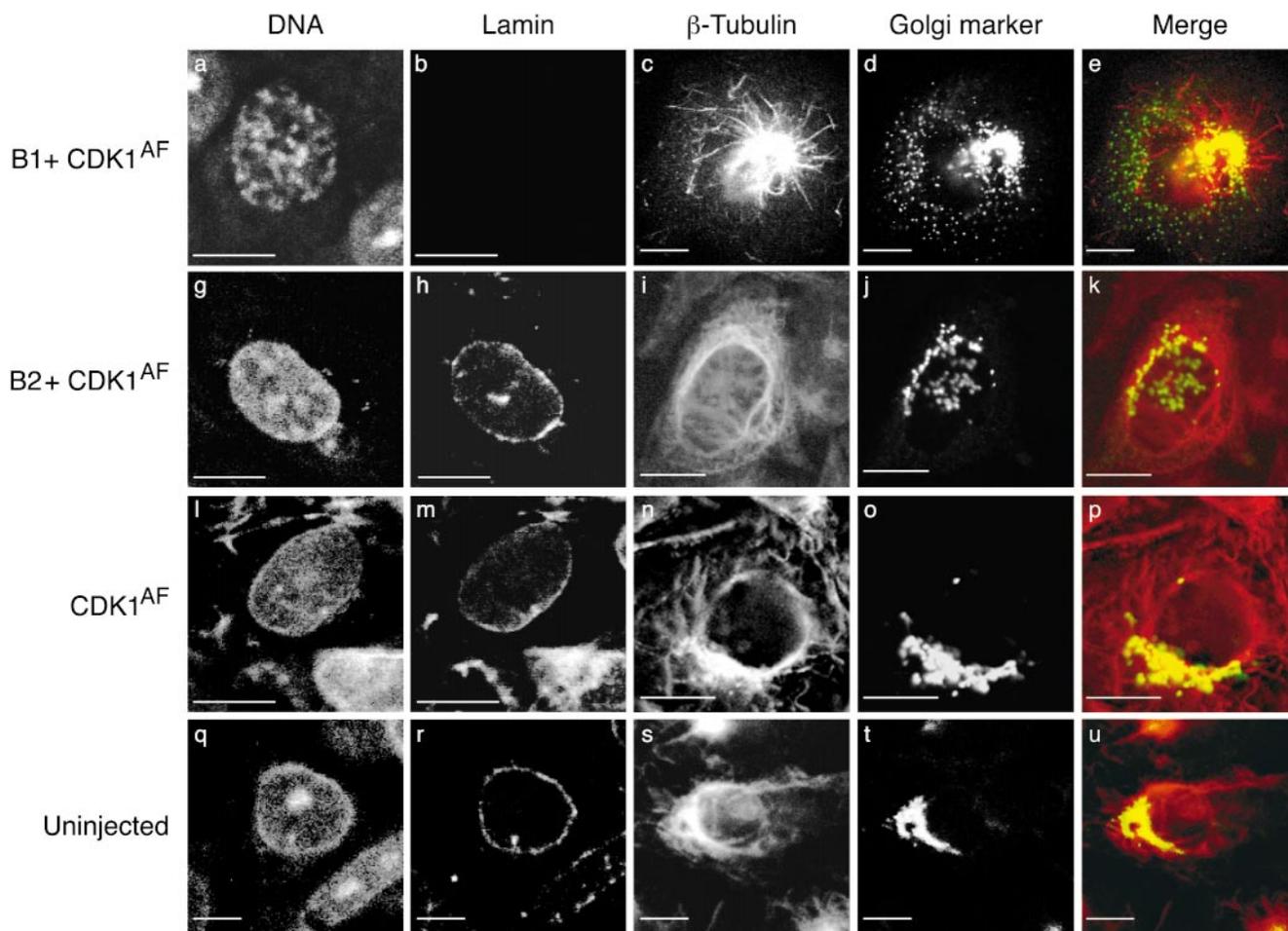


Figure 2. Different effects of cyclin B1- and B2-CDKs on subcellular architecture. Serum-starved CHO cells were unperturbed (bottom row) or microinjected with expression vectors coding for a Golgi marker NAGT-GFP (d, j, and o; green in e, k, and p) and CDK1^{AF}, alone (third row), with cyclin B1 (top row), or with cyclin B2 (second row). After 6 h, the cells were fixed and stained with TOTO-3 to visualize the DNA (a, g, l, and q) and with an antilamin antibody (b, h, m, and r) or an anti- β -tubulin antibody (c, i, n, and s; red in e, k, p, and u). Antimannosidase II was used to stain the Golgi in uninjected cells (t; green in u). Note that cyclin B1 + CDK1^{AF} caused the nuclear lamina to disassemble, and the solubilized lamin protein was washed out of the cell during fixation. The Golgi apparatus fragmented in both cyclin B1-CDK1^{AF} and cyclin B2-CDK1^{AF} injected cells, but only cyclin B1 + CDK1^{AF} caused the microtubules to become much shorter and the centrosome to nucleate more microtubules. Cells are representative of more than 120 cells analyzed in more than three separate experiments. Bars, 10 μ m.

Cyclin B1-CDK1 Reorganizes the Cytoskeleton and Chromosome Architecture but Cyclin B2-CDK1 Does Not

The striking difference in the localization of the two B-type cyclins raised the possibility that a cyclin B1-CDK1 complex might have a different substrate specificity compared with cyclin B2-CDK1. Therefore, we analyzed changes in the subcellular architecture as a consequence of expressing CDK1^{AF} with or without B-type cyclins. CDK1^{AF} alone had no effect on any component of the cells that we analyzed, further evidence that G0/G1 phase CHO cells did not contain any endogenous mitotic cyclins (Fig. 2, compare l-p and q-u). When we coexpressed CDK1^{AF} with cyclin B1, we found that the nuclear lamina was rapidly solubilized (within 3 h) in almost all the cells (Fig. 2 b; Table I). After 4 h, ~30% of cells expressing cyclin B1-CDK1^{AF} had rounded up, and in these cells the microtubules had reorganized to form a

large aster and the chromosomes had prematurely condensed (Fig. 2, a and c; Table I). After 6.5 h, almost all cells expressing cyclin B1-CDK1^{AF} had rounded up. These changes were characteristic of mitotic cells, indicating that cyclin B1-CDK1^{AF} was able, directly or indirectly, to induce mitotic changes in the nucleus and the cytoplasm. Time-lapse fluorescence studies also showed that cyclin B1-GFP translocated into the nucleus of those cells that rounded up and condensed their DNA (data not shown).

In contrast to the effects of cyclin B1-CDK1^{AF}, overexpressing cyclin B2 with CDK1^{AF} did not cause any visible changes in the chromosomes, the nuclear lamina, or the microtubules, and all the cells remained flat and well-attached to the dish (Fig. 2, g-i; Table I). These results could not be explained by different levels of expression because cyclin B1 and B2 were expressed to similar levels in these cells (Fig. 1 A) and had similar levels of associated H1 kinase activity (see below).

Table I. Effect of Different B-type Cyclin–CDKs on Subcellular Architecture

Injection	3–4 h					6–6.5 h				
	Minimum number of experiments	Disassembled Golgi	Aster-like microtubules	Solubilized lamina	Condensed chromosome	Minimum number of experiments	Disassembled Golgi	Aster-like microtubules	Solubilized lamina	Condensed chromosomes
	% (n)					% (n)				
Cyclin B1 + CDK1 ^{AF}	4	92 (89)	28 (95)	81 (59)	36 (59)	3	95 (103)	63 (70)	89 (82)	89 (82)
Cyclin B2 + CDK1 ^{AF}	4	88 (73)	0 (26)	0.6 (47)	0 (27)	3	90 (120)	1 (53)	1 (31)	1 (31)
CDK1 ^{AF}	3	15 (79)	3 (30)	2 (49)	2 (49)	2	12 (74)	6 (30)	0 (44)	0 (44)
B1–B2 chimera + CDK1 ^{AF}	3	90 (54)	39 (51)	2 (48)	2 (48)	2	93 (48)	67 (44)	0 (31)	0 (31)
B2–B1 chimera + CDK1 ^{AF}	3	88 (94)	8 (49)	2 (45)	2 (48)	2	96 (49)	0 (22)	0 (28)	0 (28)

The percentage of cells with disassembled Golgi apparatus, microtubules reorganized to form asters, solubilized nuclear lamins, and condensed chromosomes at 3–3.5 and 6–6.5 h after injection with specific cyclins and CDK constructs are given along with the number of cells scored as positive for the injection marker.

Both Cyclin B1– and Cyclin B2–CDK1 Cause the Golgi Apparatus to Disassemble

The targeting of cyclin B2 to the Golgi apparatus indicated that cyclin B2–CDK1 might be restricted to phosphorylating components of the Golgi. Therefore, we assayed the integrity of the Golgi apparatus by two different means. First, we used an antimannosidase II or antigiantin antibody as a marker for the Golgi apparatus in immunofluorescence analysis. Second, we used GFP fused to the Golgi retention signal of NAGT–GFP (a gift from G. Warren, Yale University, New Haven, CT) (Shima et al., 1997) to analyze changes in the Golgi apparatus by time-lapse fluorescence microscopy.

Expressing CDK1^{AF} alone had no effect on the integrity of the Golgi apparatus in G0/G1 phase CHO cells as judged by either antimannosidase II or NAGT–GFP staining (Fig. 2, o). When we coexpressed cyclin B1 with CDK1^{AF}, the Golgi broke down and took on an appearance similar to that seen in early metaphase (Fig. 2, d and e; Table I). In marked contrast to its lack of effect on the nucleus and the microtubules, coexpressing cyclin B2 with CDK1^{AF} also caused the Golgi to disperse (Fig. 2, j and k; Table I). Using three different Golgi markers, we noticed that cyclin B2–CDK1^{AF} tended to cause the Golgi to disperse into fewer and larger vesicles than cyclin B1–CDK1^{AF} (NAGT–GFP and antigiantin shown in Fig. 3; antimannosidase, not shown). This could not be ascribed to changes in microtubule organization instigated by cyclin B1–CDK1^{AF} because it was also seen in cells expressing cyclin B1–CDK1^{AF} in which the microtubule array was unchanged (Fig 3, c, e, and g).

The NH₂ Terminus of the B-type Cyclins Determines the Subcellular Localization and Substrates of the CDK

Our results indicated that cyclin B2 restricted the substrate specificity of CDK1 to Golgi-associated proteins by targeting it to the Golgi apparatus. However, an alternative explanation could be that the hydrophobic patch region of cyclin B2 was only able to bind a restricted set of substrates, although this appeared unlikely because there were only a few changes between cyclin B1 and B2 in this region. Nevertheless, we constructed a series of chimeras between cyclin B1 and B2 to identify the region that targeted cyclin B2 to the Golgi apparatus. We generated chimeras in which the exchange between the cyclins was made at conserved motifs in the two cyclins. This showed that amino acids 1–127 were required to target

cyclin B2 to the Golgi apparatus (Fig. 4 A). Replacing the NH₂ terminus of cyclin B1 with this region of cyclin B2 (Fig. 4 A) targeted cyclin B1 to the Golgi apparatus (Fig. 4 B). Importantly, this chimera contained the whole of the cyclin box and second cyclin fold of cyclin B1, including the hydrophobic patch of cyclin B1 and was able to bind and activate CDK1 to a similar extent as wild-type cyclin B1 (Fig. 4 D). When we coexpressed the B2–B1 chimera with CDK1^{AF}, it was now only able to cause the Golgi apparatus to disassemble (Fig. 4 C). It did not cause chromosome condensation or nuclear lamina breakdown (Fig. 4 C), nor did it alter the microtubule array or translocate to the nucleus in any of the cells that we assayed.

From these results, we predicted that the converse chimera, one where the cyclin box and second cyclin fold of cyclin B2 was targeted to the cytoplasm by the NH₂ terminus of cyclin B1, would confer on cyclin B2 the broader substrate specificity of cyclin B1. This prediction was confirmed when we coexpressed a B1–B2 chimera (Fig. 5 A) with CDK1^{AF} and found that it was localized throughout the cytoplasm and not restricted to the Golgi apparatus (Fig. 5 B). This chimera also bound and activated CDK1 to a similar extent as the wild-type protein (Fig. 4 D). Although the chimera had the hydrophobic patch and cyclin folds of cyclin B2, it was able to cause the microtubules to reorganize in addition to causing Golgi disassembly (Fig. 5 C). However, the chimera was not able to shuttle into the nucleus in the presence of LMB (data not shown). In agreement with its restricted cytoplasmic localization, it had no effect on either the condensation state of the DNA or the integrity of the nuclear lamina (Fig. 5 C).

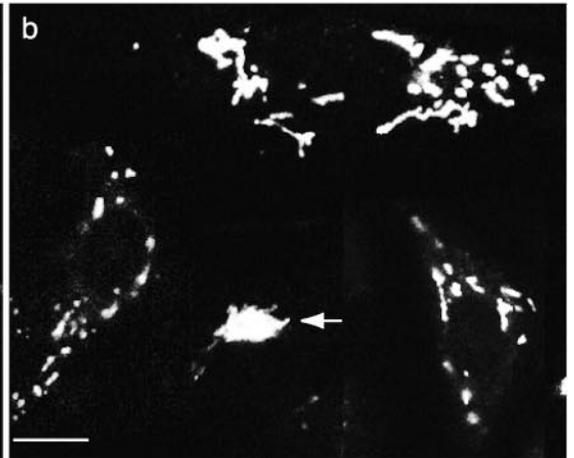
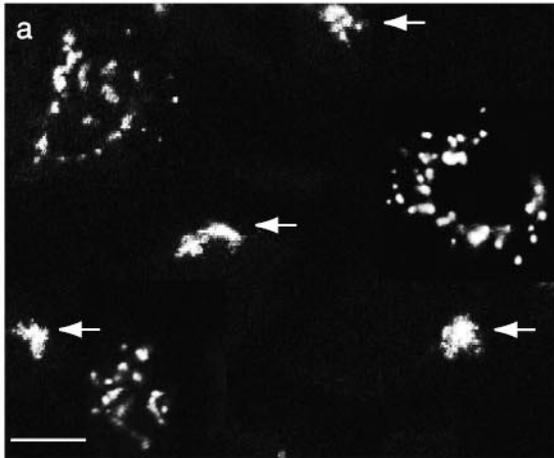
B-type Cyclin–CDK1 Kinases Do Not Require MEK to Disassemble the Golgi Apparatus

Time-lapse fluorescence microscopy using NAGT–GFP showed that both cyclin B1– and cyclin B2–CDK1 complexes caused the Golgi to begin to disassemble at approximately the same time after microinjection (data not shown). This raised the question of whether B-type cyclin–CDK complexes required MEK to cause Golgi disassembly. In our microinjection regime, we used serum-starved cells to which we added 2% serum to aid recovery from the microinjection before analyzing them 3 h later. Although this treatment did not generate any endogenous mitotic cyclins over the time course of our experiments (Fig. 1 C), it did activate MEK (Fig. 6, A and B). However, none of the

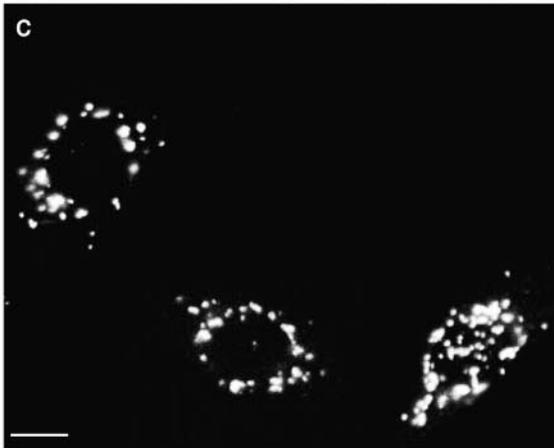
B1+ CDK1^{AF}

B2 + CDK1^{AF}

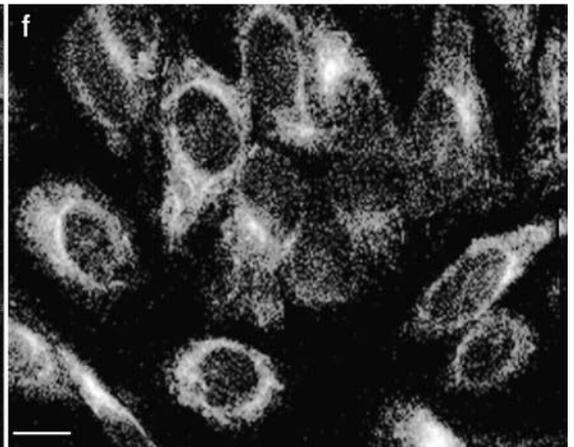
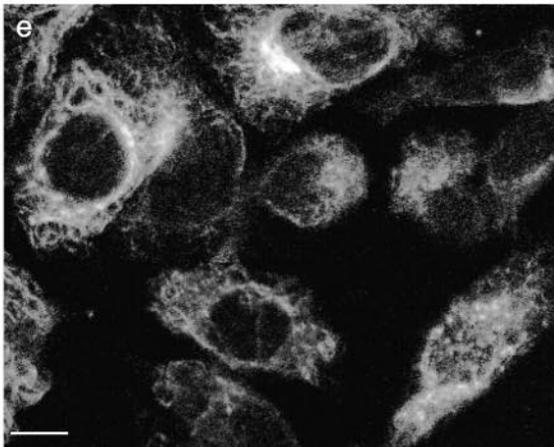
Giantin



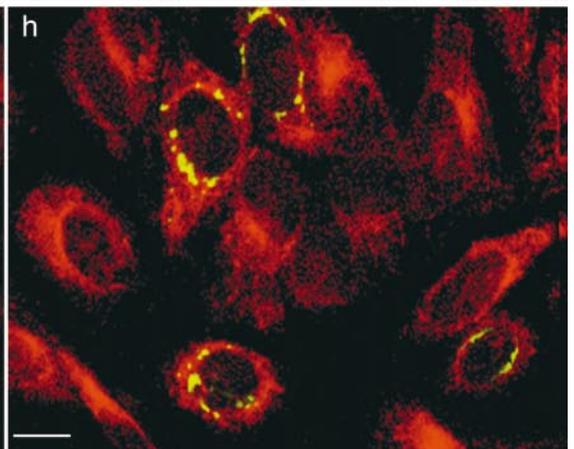
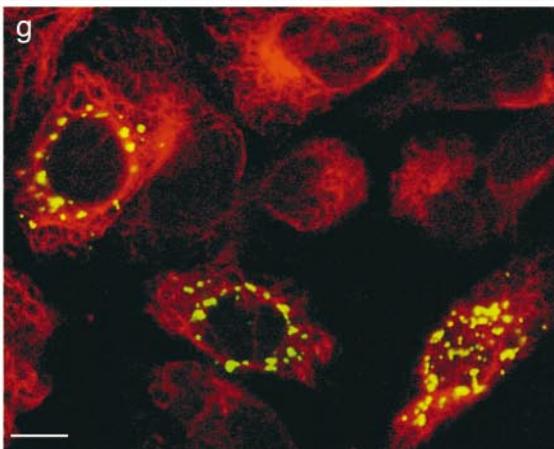
NAGT-GFP



β -Tubulin



Merge



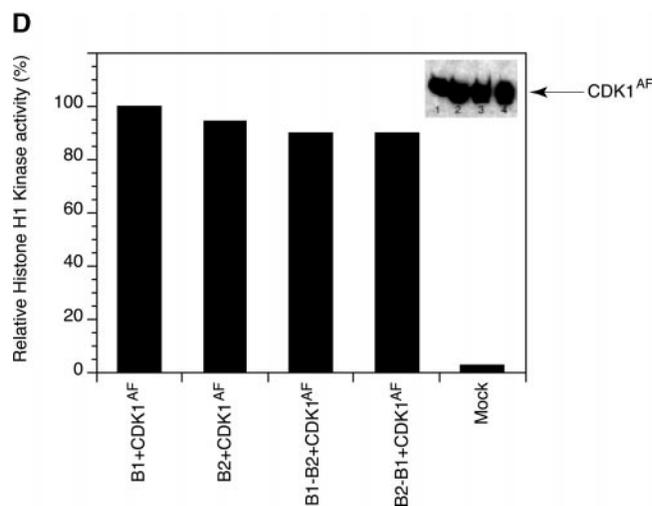
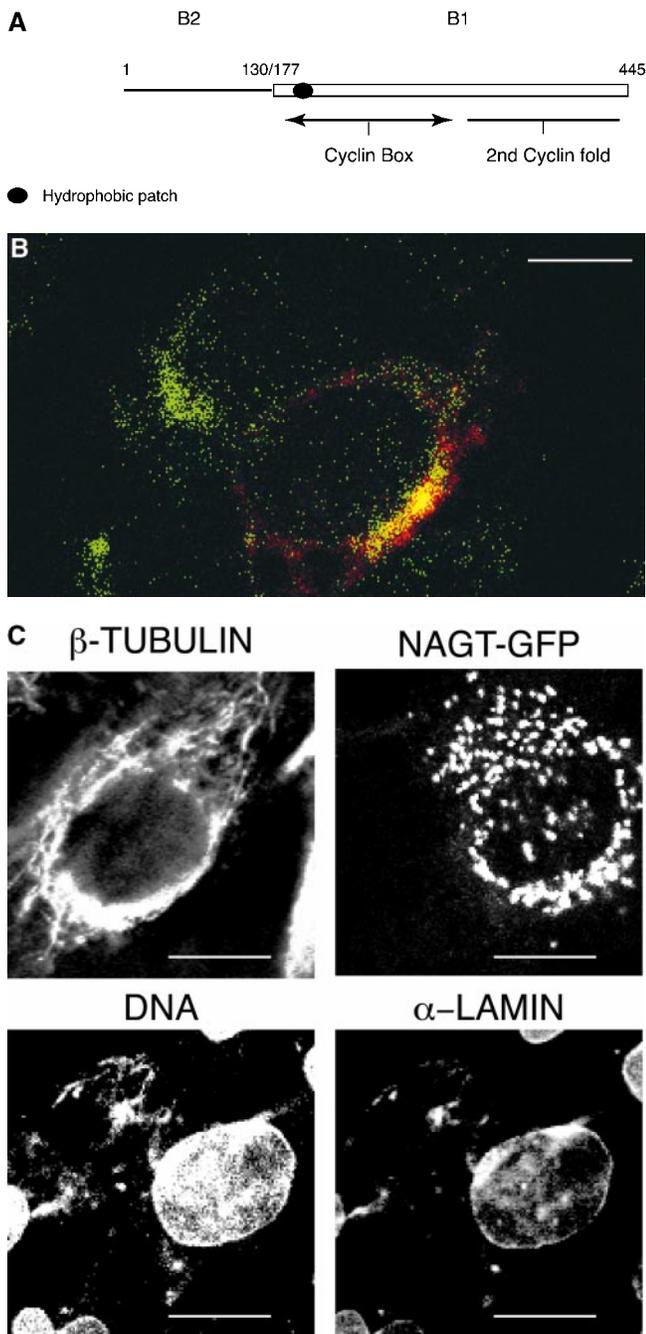


Figure 4. The NH₂ terminus of cyclin B2 targets cyclin B1 to the Golgi and restricts its activity. (A) Schematic diagram of the chimera constructed between cyclin B2 and cyclin B1. Cyclin B2 is represented by a solid line and cyclin B1 by an open rectangle. The cyclin B2–B1 mutant exchanges at the sequence LCS (S¹³⁰ in cyclin B2, and S¹⁷⁷ in cyclin B1) in both cyclins. The hydrophobic patch is represented by the filled oval. (B) The NH₂ terminus of cyclin B2 targets cyclin B1 to the Golgi apparatus. Serum-starved CHO cells were microinjected with expression vectors coding for a Golgi marker NAGT–GFP (green) and with a myc epitope–tagged cyclin B2–B1 chimera. After 6 h, the cells were fixed and stained with an anti-myc epitope antibody (red). One uninjected cell and one cell expressing the chimera are shown. (C) The NH₂ terminus of cyclin B2 confers the properties of cyclin B2 on cyclin B1. Serum-starved CHO cells were microinjected with expression vectors coding for a Golgi marker NAGT–GFP and CDK1^{AF} with a cyclin B2–B1 chimera. After 6 h, the cells were fixed and stained with an anti-β-tubulin antibody or with an anti-lamin antibody and TOTO-3 to visualize the DNA. Cells are representative of >100 cells analyzed in three separate experiments. (D) Cyclin B chimeras bind and activate CDK1 to a similar extent as wild-type cyclins. Human 293T cells were mock transfected with an empty vector or transfected with untagged CDK1^{AF} and myc epitope–tagged cyclins B1, B2, or the B1–B2 or B2–B1 chimera. 12 h after transfection, cells were lysed with NP-40 lysis buffer and the transfected cyclins were immunoprecipitated with an anti-myc epitope antibody. Immunoprecipitates were processed for H1 kinase assays and the amount of phosphate incorporated was quantitated and normalized to the H1 kinase activity in the cyclin B1–CDK1 sample. In parallel, immunoprecipitates were immunoblotted with an anti-CDK1 monoclonal (inset) and an anti-myc epitope antibody (not shown) to demonstrate that equivalent amounts of cyclins were immunoprecipitated. Results shown are representative of two independent experiments. Bars, 10 μm.

cells disassembled their Golgi apparatus in the absence of mitotic cyclin–CDKs (Fig. 2, o and t).

To analyze the effect of the mitotic cyclin–CDKs in the absence of active MEK, we treated serum-starved cells with the MEK kinase inhibitors PD98059 (20 μM) and U0126 (25

μM) for 1 h before microinjection and continued to incubate cells with these inhibitors after microinjection. We assayed MEK activity by immunoblotting cell lysates with an anti-phospho-MAP kinase antibody that only recognized MAP kinases that had been phosphorylated by MEK (Fig. 6 A).

Figure 3. Cyclin B1–CDK1^{AF} and cyclin B2–CDK1^{AF} have different effects on the Golgi apparatus. Serum-starved CHO cells were microinjected with expression vectors coding for a Golgi marker NAGT–GFP (c and d) and cyclin B1 with CDK1^{AF} (a, c, e, and g) or cyclin B2 with CDK1^{AF} (b, d, f, and h). Cells were fixed and stained with antimannosidase II (not shown) and antigiantin (a and b, arrows denote uninjected cells) or with an anti-β-tubulin antibody (e and f). The merge shown (g and h) is between antitubulin and NAGT–GFP. Note that cyclin B1–CDK1^{AF} causes the Golgi to break down into smaller and more numerous vesicles than does cyclin B2–CDK1^{AF} in cells before observable changes in the cytoskeleton. Cells are representative of >250 cells analyzed in >10 separate experiments. Bars, 10 μm.

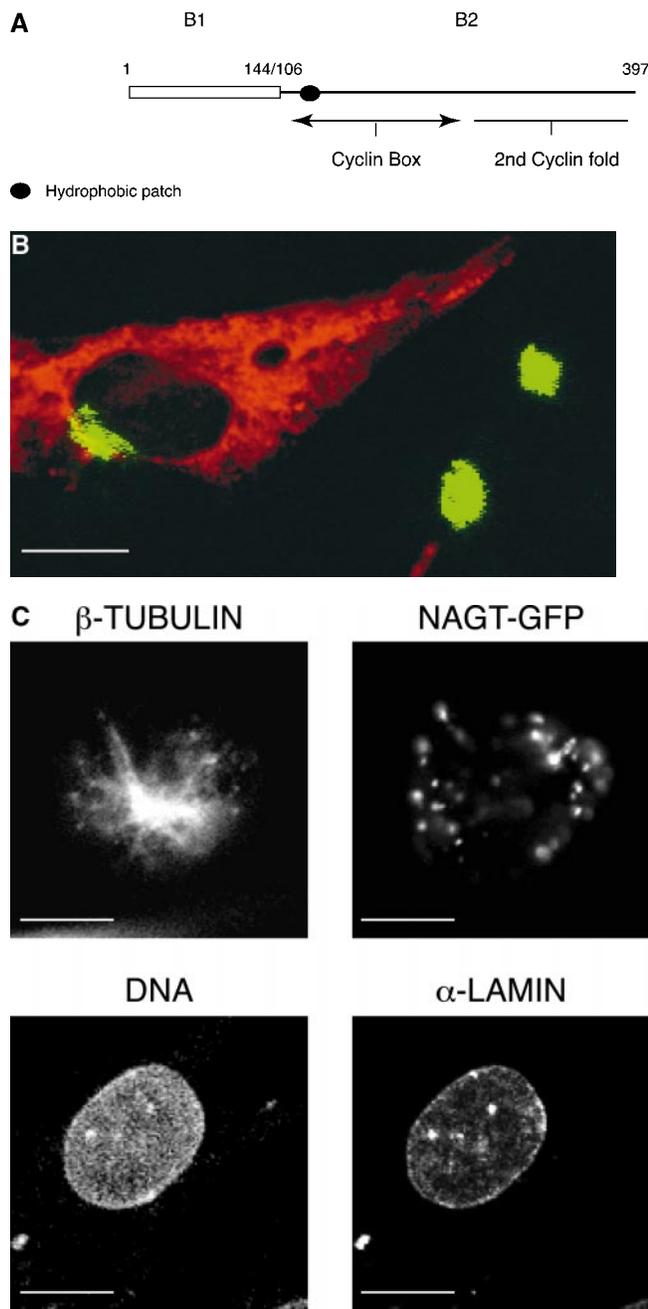


Figure 5. The NH₂ terminus of cyclin B1 targets cyclin B2 to the cytoplasm and broadens its activity. (A) Schematic diagram of the chimera constructed between cyclin B1 and cyclin B2. Cyclin B1 is represented by an open rectangle and cyclin B2 by a solid line. The cyclin B1–B2 mutant exchanges at the sequence LCQ in both cyclins (Q¹⁰⁶ in B1, and Q¹⁴⁴ in B2). (B) The NH₂ terminus of cyclin B1 targets cyclin B2 to the cytoplasm. Serum-starved CHO cells were microinjected with expression vectors coding for a Golgi marker NAGT–GFP (green) and with a myc epitope–tagged cyclin B1–B2 chimera. After 6 h, the cells were fixed and stained with an anti-myc epitope antibody (red). (C) The NH₂ terminus of cyclin B1 confers some of the properties of cyclin B1 on cyclin B2. Serum-starved CHO cells were microinjected with expression vectors coding for a Golgi marker NAGT–GFP and CDK1^{AF} with a cyclin B1–B2 chimera. After 6 h, the cells were fixed and stained with an anti- β -tubulin antibody or with an antilamin antibody and TOTO-3. Cells are representative of >150 cells analyzed in three separate experiments. Bars, 10 μ m.

As a loading control, we immunoblotted the same extracts with anti-ERK1 and anti-ERK2 antibodies that recognized both the phosphorylated and the unphosphorylated forms of ERK1 and ERK2 (Fig. 6 B). Incubating cells with U0126 and PD98059 prevented any phosphorylation of MAP kinase when 2% serum was added back to serum-starved cells, showing that MEK activity was inhibited (Fig. 6 A). Nevertheless, microinjecting cyclin B1–CDK1^{AF} or cyclin B2–CDK1^{AF} into U0126- and PD98059-treated cells caused the Golgi apparatus to disassemble with similar efficiency compared with cells incubated without MEK inhibitors (Fig. 6 C). Thus, MEK activity was not required for mitotic cyclin–CDKs to break down the Golgi apparatus in living cells. Furthermore, the Golgi disassembled by a similar mechanism to that seen in mitosis because it was recognized by a polyclonal antibody directed against the Ser25-phosphorylated form of the Golgi matrix protein GM130 (Lowe et al., 2000). GM130 had been shown to be phosphorylated on Ser25 only in mitosis, and this phosphorylation was required for Golgi disassembly (Lowe et al., 1998, 2000). Both cyclin B1–CDK1^{AF} and cyclin B2–CDK1^{AF} generated the phospho-Ser25 form of GM130 in the presence or absence of MEK activity, and this was coincident with Golgi disassembly (Fig. 6 C). Activating MEK by adding back serum in the absence of any mitotic cyclin–CDKs did not generate Ser25-phosphorylated GM130 (data not shown).

We also excluded the possibility that MEK was required to break down the Golgi apparatus during normal mitosis, that is, in cells with endogenous levels of B-type cyclin–CDK activity. We treated G2 phase CHO cells with the same concentrations of U0126 and PD98059 that we used for serum-stimulated cells and found that this inhibited MEK in G2 phase cells (Fig. 7, A and B). These cells proceeded through mitosis at the same rate as untreated cells (Draviam, V.M., and J. Pines, manuscript in preparation), and there was no difference between control cells and MEK-inhibited cells in disassembly of the Golgi apparatus as analyzed by confocal immunofluorescence microscopy (Fig. 7 C). These data showed that neither MEK, ERK1, nor ERK2 was required for the disassembly of the Golgi apparatus in normal mitosis.

Discussion

Subcellular Localization Has a Profound Influence on the Substrates of Mitotic Cyclin–CDKs

In this paper, we show that subcellular localization profoundly affects the substrate specificity of B-type cyclin–CDK complexes. We have shown that CDK1 activated by human cyclin B1 is able to reorganize nuclear, cytoskeletal, and membrane compartments in the cell, whereas when activated by cyclin B2 it is only able to disassemble the Golgi apparatus. Furthermore, directing cyclin B1 to the Golgi apparatus restricts its ability to effect all but the reorganization of the Golgi apparatus. Equally, if cyclin B2–CDK1^{AF} is liberated from the Golgi apparatus, it is able to reorganize the cytoskeleton in a similar manner to wild-type cyclin B1–CDK1^{AF}. Thus, the primary control on substrate selection by the mitotic cyclins is their subcellular localization, and this is distinct from any substrate-binding role of the hydrophobic patch of the cyclin box.

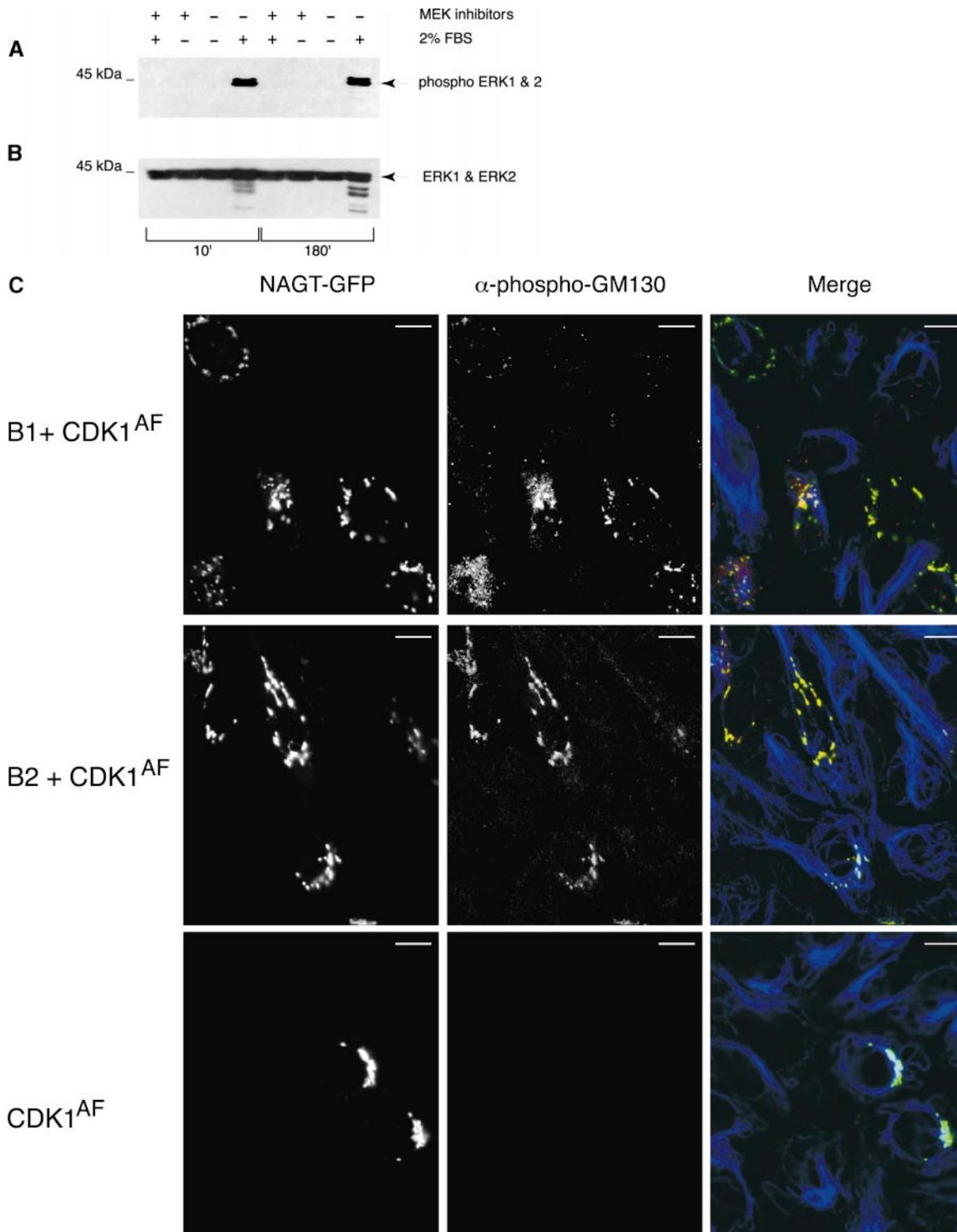


Figure 6. Cyclin B–CDK1 complexes will disassemble the Golgi apparatus in a physiologically relevant manner in the absence of MEK activity. (A and B) CHO cells were incubated in DME containing 0.1% serum for 3 d (lanes 3 and 7), after which DME with 2% FCS was added back in the presence (lanes 1 and 5) or absence (lanes 4 and 8) of the MEK inhibitors, U0126 (25 μ M) and PD98059 (20 μ M). MEK inhibitors were also added to cells in the absence of serum (lanes 2 and 6). Cells were lysed after 10 min (lanes 1–4) or 3 h (lanes 5–8), and whole cell extracts were probed with an anti-phospho-MAP kinase antibody (A) and subsequently reprobbed with an antibody that recognizes all forms of MAP kinase (B) as a loading control. Results shown are representative of three independent experiments. (C) CHO cells were incubated in DME containing 0.1% serum for 3 d and then microinjected with expression constructs for NAGT-GFP (left; and green, in right panels) with either cyclin B1 and CDK1^{AF} (top panels), cyclin B2 and CDK1^{AF} (middle panels), or CDK1^{AF} (bottom panels) in the presence of 2% serum and MEK inhibitors as in A and B. After 3 h, cells were fixed and stained with an antibody that recognizes the phosphorylated form of GM130 (center; and red, in right panels) and an anti- β -tubulin antibody (blue in right panels). Results shown are representative of two independent experiments. Bars, 10 μ m.

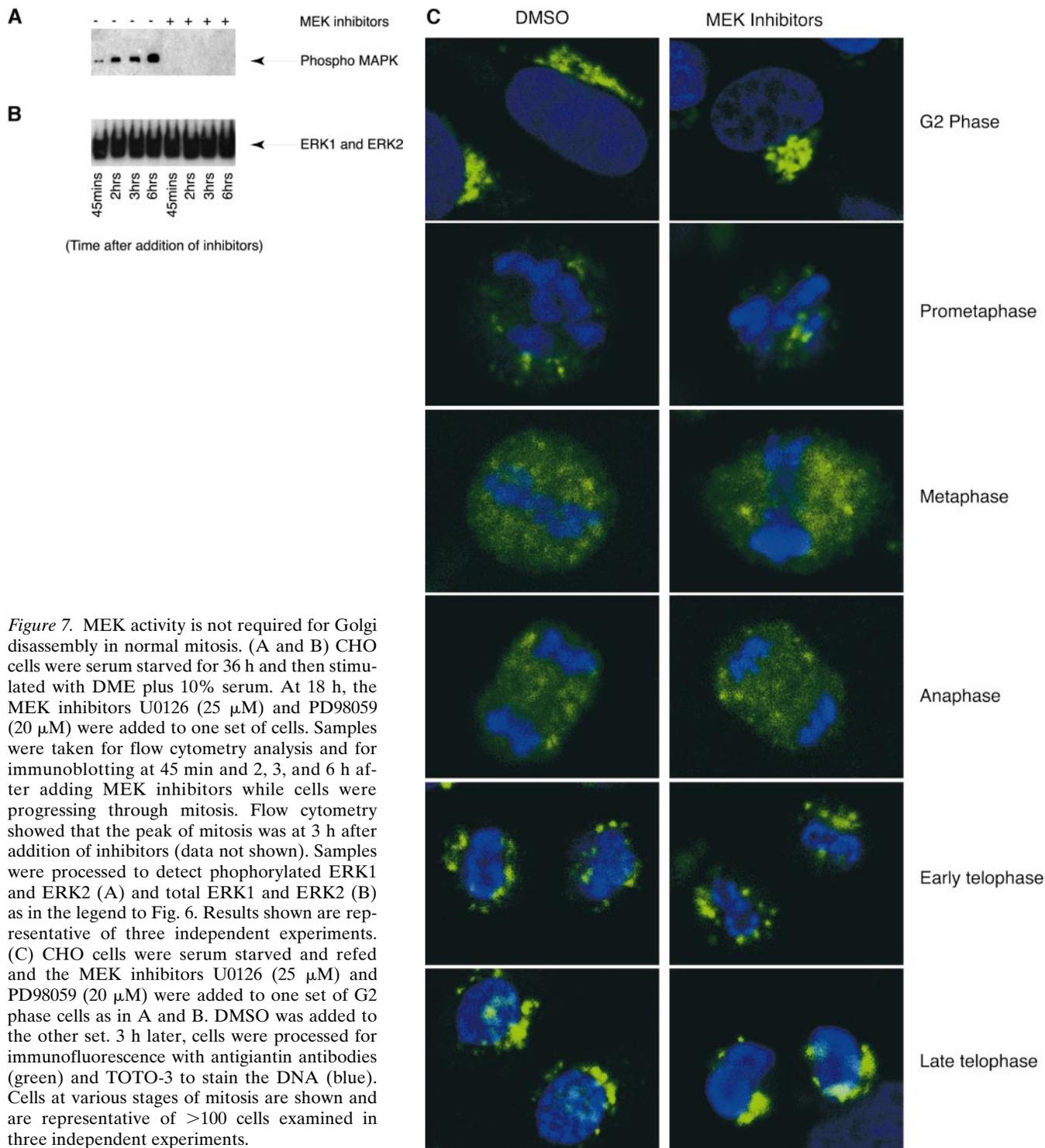


Figure 7. MEK activity is not required for Golgi disassembly in normal mitosis. (A and B) CHO cells were serum starved for 36 h and then stimulated with DME plus 10% serum. At 18 h, the MEK inhibitors U0126 (25 μ M) and PD98059 (20 μ M) were added to one set of cells. Samples were taken for flow cytometry analysis and for immunoblotting at 45 min and 2, 3, and 6 h after adding MEK inhibitors while cells were progressing through mitosis. Flow cytometry showed that the peak of mitosis was at 3 h after addition of inhibitors (data not shown). Samples were processed to detect phosphorylated ERK1 and ERK2 (A) and total ERK1 and ERK2 (B) as in the legend to Fig. 6. Results shown are representative of three independent experiments. (C) CHO cells were serum starved and refed and the MEK inhibitors U0126 (25 μ M) and PD98059 (20 μ M) were added to one set of G2 phase cells as in A and B. DMSO was added to the other set. 3 h later, cells were processed for immunofluorescence with anti-giantin antibodies (green) and TOTO-3 to stain the DNA (blue). Cells at various stages of mitosis are shown and are representative of >100 cells examined in three independent experiments.

Shortly after expressing cyclin B1-CDK1^{AF}, only a minority of G0/G1 cells have reorganized microtubules and condensed their DNA, but almost all have disassembled the Golgi apparatus and solubilized the nuclear lamina. This may indicate that cyclin B1-CDK1 has a greater affinity for the nuclear lamins and for Golgi apparatus substrates than for chromosomal or microtubule-associated proteins. Alternatively, there may be additional factors required for chromosome condensation and microtubule disassembly that are limiting in G0/G1 phase cells.

Surprisingly, we find that the nuclear membrane remains intact (as judged by differential interference contrast microscopy) in cells where the nuclear lamins have been solubilized by cyclin B1-CDK1. Therefore, cyclin B1-CDK1 does not appear to be the protein kinase responsible for nuclear envelope, as opposed to nuclear lamina breakdown in agreement with studies on nuclear membrane assembly and disassembly *in vitro* (Pfaller et al., 1991). The ability of cyclin B1-CDK1^{AF} to phosphorylate both nuclear and cytoplasmic substrates is likely to be be-

cause it can shuttle between the nucleus and the cytoplasm during interphase. In contrast, neither cyclin B2 nor the cyclin B1–B2 chimera are able to alter the nuclear architecture, and this correlates with their inability to enter the nucleus in the presence of LMB.

Thus, there is a dramatic difference in the biological action of cyclins B1 and B2 even though both are targeted to the cytoplasm. Miller and Cross (2000) showed that redirecting a nuclear cyclin, a truncation mutant of the budding yeast Cln3p, to the cytoplasm with an exogenous nuclear export sequence perturbed its ability to complement a strain deficient in CLN3. They also showed that this mutant protein appeared to take on some of the properties of the cytoplasmic Cln2 (Miller and Cross, 2000). We have shown here that it is also important where in the cytoplasm the cyclin is directed. Subcellular localization could also have an important role in the specialization of different nuclear cyclin–CDKs that may have been overlooked, especially given that the nucleus is composed of distinct regions and structures to which proteins are specifically targeted (for review see Lamond and Earnshaw, 1998). Thus, one can envisage several specific subcellular sites where protein kinases, phosphatases, and other regulators integrate intracellular signals in functional isolation from the rest of the cell. These sites of signal integration would eventually have to be coupled to each other to coordinate entry into mitosis. This could most obviously be achieved by relocalizing an activated enzyme from one subcellular compartment to another, for example, by the translocation of cyclin B1–CDK1 to the nucleus at the end of prophase.

Similarly, it is important to take into account the localization of ectopically expressed proteins when interpreting studies that appear to show complementation between cyclins. For example, Clb2 is able to perform the functions of Clb5 when it is overexpressed from the strong GAL promoter but not when it is driven by the much weaker Clb5 promoter (Cross et al., 1999). This effect was interpreted as evidence in favor of the specificity of substrate selection by the hydrophobic patch of Clb5, but it might also be explained by excess Clb2 being able to localize to late origins of DNA replication that are normally targeted by Clb5. In fission yeast, the observation that low levels of *cdc13/cdc2* drive S phase and high levels drive M phase (Stern and Nurse, 1996) could be explained should higher levels of *cdc13/cdc2* bind to low affinity sites where they encounter M phase substrates.

B-type Cyclin–CDKs Can Disassemble the Golgi Apparatus without MEK

We find that cyclin B1–CDK1 and cyclin B2–CDK1 are equally able to initiate Golgi disassembly, although neither kinase alone is able to reproduce the complete disassembly observed in mitotic cells. However, the breakdown of the Golgi apparatus induced by cyclin B1–CDK1 in G0/G1 phase is accompanied by the phosphorylation of GM130 on Ser25 that is characteristic of Golgi disassembly in mitosis. When GM130 is phosphorylated on Ser25, vesicles continue to be shed from the Golgi but cannot bind again because the vesicle protein p115 cannot bind phospho-GM130 (Lowe et al., 1998, 2000). Ser25 of GM130 can be directly phosphorylated *in vitro* by cyclin B1– and cyclin B2–CDK1 complexes (Lowe et al., 1998).

The nature of the other proteins required to effect complete Golgi disassembly is unclear but they do not appear to include MEK because we have shown here that actively dividing cells disassemble their Golgi apparatus in the presence of the MEK inhibitors, U0126 and PD98059.

Warren and colleagues (Misteli and Warren, 1995a; Lowe et al., 1998, 2000) have concluded that B-type cyclin–CDKs, rather than the Golgi-associated MEK1 postulated by Malhotra and colleagues (Acharya et al., 1998; Colanzi et al., 2000; Kano et al., 2000), are responsible for Golgi fragmentation at mitosis. Our results support this conclusion. GM130 was phosphorylated on Ser25 in cells expressing either cyclin B1– or B2–CDK1^{AF}, and there was no difference in the ability of either B-type cyclin–CDK1 to cause Golgi disassembly, or to phosphorylate GM130, in the presence or absence of the MEK inhibitors U0126 and PD98059. These inhibitors also have no effect on the breakdown of the Golgi apparatus in cells undergoing normal mitosis. Nor do our results support the model that MEK activity is required for the initial stages of Golgi disassembly and cyclin–CDK activity for subsequent steps (Kano et al., 2000). One difference between our analysis and the studies that have implicated a role for MEK in mitotic Golgi disassembly is that we have analyzed disassembly in intact, living cells rather than *in vitro* systems using permeabilized cells or purified membranes added to cytosol. Because we have not salt-treated or permeabilized our cells, we have avoided the possibility that protein kinases may be able to phosphorylate substrates with which they do not normally interact.

Our results demonstrate that localization profoundly influences substrate selection by mitotic cyclin–CDKs. This may also be extrapolated to other protein kinase families that show specific subcellular localizations. In addition, protein kinases that exhibit dynamic changes in their subcellular localization could perform different functions at different times depending on where they are in the cell (for review see Pines, 1999). Clearly, a complete understanding of the function of a protein kinase must take account of both when it is active and where it is localized.

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References

- Acharya, U., A. Mallabiabarrena, J.K. Acharya, and V. Malhotra. 1998. Signaling via mitogen-activated protein kinase kinase (MEK1) is required for Golgi fragmentation during mitosis. *Cell*. 92:183–192.
- Brandeis, M., and T. Hunt. 1996. The proteolysis of mitotic cyclins in mamma-

- lian cells persists from the end of mitosis until the onset of S phase. *EMBO (Eur. Mol. Biol. Organ.) J.* 15:5280–5289.
- Brandeis, M., I. Rosewell, M. Carrington, T. Crompton, M.A. Jacobs, J. Kirk, J. Gannon, and T. Hunt. 1998. Cyclin B2-null mice develop normally and are fertile whereas cyclin B1-null mice die *in utero*. *Proc. Natl. Acad. Sci. USA.* 95:4344–4349.
- Chapman, D.L., and D.J. Wolgemuth. 1993. Isolation of the murine cyclin B2 cDNA and characterization of the lineage and temporal specificity of expression of the B1 and B2 cyclins during oogenesis, spermatogenesis and early embryogenesis. *Development.* 118:229–240.
- Chen, J., P.K. Jackson, M.W. Kirschner, and A. Dutta. 1995. Separate domains of p21 involved in the inhibition of cdk kinase and PCNA. *Nature.* 374:386–388.
- Clute, P., and J. Pines. 1999. Temporal and spatial control of cyclin B1 destruction in metaphase. *Nat. Cell Biol.* 1:82–87.
- Colanzi, A., T.J. Deerinck, M.H. Ellisman, and V. Malhotra. 2000. A specific activation of the mitogen-activated protein kinase kinase 1 (MEK1) is required for Golgi fragmentation during mitosis. *J. Cell Biol.* 149:331–339.
- Cross, F.R., and M.D. Jacobson. 2000. Conservation and function of a potential substrate-binding domain in the yeast Clb5 B-type cyclin. *Mol. Cell. Biol.* 20:4782–4790.
- Cross, F.R., M. Yuste-Rojas, S. Gray, and M.D. Jacobson. 1999. Specialization and targeting of B-type cyclins. *Mol. Cell.* 4:11–19.
- Fisher, D.L., and P. Nurse. 1996. A single fission yeast mitotic cyclin B p34cdc2 kinase promotes both S phase and mitosis in the absence of G1 cyclins. *EMBO (Eur. Mol. Biol. Organ.) J.* 15:850–860.
- Furuno, N., N. den Elzen, and J. Pines. 1999. Human cyclin A is required for mitosis until mid prophase. *J. Cell Biol.* 147:295–306.
- Geng, Y., W. Whoriskey, M.Y. Park, R.T. Bronson, R.H. Medema, T. Li, R.A. Weinberg, and P. Sicinski. 1999. Rescue of cyclin D1 deficiency by knockin cyclin E. *Cell.* 97:767–777.
- Hagting, A., C. Karlsson, P. Clute, M. Jackman, and J. Pines. 1998. MPF localization is controlled by nuclear export. *EMBO (Euro. Mol. Biol. Organ.) J.* 17:4127–4138.
- Hagting, A., M. Jackman, K. Simpson, and J. Pines. 1999. Translocation of cyclin B1 to the nucleus at prophase requires a phosphorylation-dependent nuclear import signal. *Curr. Biol.* 9:680–689.
- Heintz, N., H.L. Sive, and R.G. Roeder. 1983. Regulation of human histone gene expression: kinetics of accumulation and changes in the rate of synthesis and in the half-lives of individual histone mRNAs during the HeLa cell cycle. *Mol. Cell. Biol.* 3:539–550.
- Jackman, M., M. Firth, and J. Pines. 1995. Human cyclins B1 and B2 are localized to strikingly different structures: B1 to microtubules, B2 primarily to the Golgi apparatus. *EMBO (Eur. Mol. Biol. Organ.) J.* 14:1646–1654.
- Jacobson, M.D., S. Gray, M. Yuste-Rojas, and F.R. Cross. 2000. Testing cyclin specificity in the exit from mitosis. *Mol. Cell. Biol.* 20:4483–4493.
- Jeffrey, P.D., A.A. Russo, K. Polyak, E. Gibbs, J. Hurwitz, J. Massague, and N.P. Pavletich. 1995. Structure of a cyclin A-CDK2 complex. *Nature.* 376:313–320.
- Kano, F., K. Takenaka, A. Yamamoto, K. Nagayama, E. Nishida, and M. Murata. 2000. MEK and Cdc2 kinase are sequentially required for Golgi disassembly in MDCK cells by the mitotic *Xenopus* extracts. *J. Cell Biol.* 149:357–368.
- Lamond, A.I., and W.C. Earnshaw. 1998. Structure and function in the nucleus. *Science.* 280:547–553.
- Lowe, M., C. Rabouille, N. Nakamura, R. Watson, M. Jackman, E. Jamsa, D. Rahman, D.J. Pappin, and G. Warren. 1998. Cdc2 kinase directly phosphorylates the cis-Golgi matrix protein GM130 and is required for Golgi fragmentation in mitosis. *Cell.* 94:783–793.
- Lowe, M., N.K. Gonatas, and G. Warren. 2000. The mitotic phosphorylation cycle of the cis-Golgi matrix protein GM130. *J. Cell Biol.* 149:341–356.
- Miller, M.E., and F.R. Cross. 2000. Distinct subcellular localization patterns contribute to functional specificity of the Cln2 and Cln3 cyclins of *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* 20:542–555.
- Misteli, T., and G. Warren. 1995a. Mitotic disassembly of the Golgi apparatus *in vivo*. *J. Cell Sci.* 108:2715–2727.
- Misteli, T., and G. Warren. 1995b. A role for tubular networks and a COP I-independent pathway in the mitotic fragmentation of Golgi stacks in a cell-free system. *J. Cell Biol.* 130:1027–1039.
- Morgan, D.O. 1995. Principles of CDK regulation. *Nature.* 374:131–134.
- Morgan, D.O. 1997. Cyclin-dependent kinases: engines, clocks, and microprocessors. *Annu. Rev. Cell Dev. Biol.* 13:261–291.
- Nelson, W.J. 2000. W(h)ither the Golgi during mitosis? *J. Cell Biol.* 149:243–248.
- Noble, M.E., J.A. Endicott, N.R. Brown, and L.N. Johnson. 1997. The cyclin box fold: protein recognition in cell-cycle and transcription control. *Trends Biochem. Sci.* 22:482–487.
- Pfaller, R., C. Smythe, and J.W. Newport. 1991. Assembly/disassembly of the nuclear envelope membrane: cell cycle-dependent binding of nuclear membrane vesicles to chromatin *in vitro*. *Cell.* 65:209–217.
- Piaggio, G., A. Farina, D. Perrotti, I. Manni, P. Fuschi, A. Sacchi, and C. Gaetano. 1995. Structure and growth-dependent regulation of the human cyclin B1 promoter. *Exp. Cell Res.* 216:396–402.
- Pines, J. 1995. Cyclins and cyclin-dependent kinases: theme and variations. *Adv. Cancer Res.* 66:181–212.
- Pines, J. 1999. Four-dimensional control of the cell cycle. *Nat. Cell Biol.* 1:E73–E79.
- Pines, J., and T. Hunter. 1991. Human cyclins A and B are differentially located in the cell and undergo cell cycle dependent nuclear transport. *J. Cell Biol.* 115:1–17.
- Roberts, J.M. 1999. Evolving ideas about cyclins. *Cell.* 98:129–132.
- Russo, A.A., P.D. Jeffrey, A.K. Patten, J. Massague, and N.P. Pavletich. 1996. Crystal structure of the p27Kip1 cyclin-dependent-kinase inhibitor bound to the cyclin A-Cdk2 complex. *Nature.* 382:325–331.
- Saha, P., Q. Eichbaum, E.D. Silberman, B.J. Mayer, and A. Dutta. 1997. p21CIP1 and Cdc25A: competition between an inhibitor and an activator of cyclin-dependent kinases. *Mol. Cell. Biol.* 17:4338–4345.
- Schulman, B.A., D.L. Lindstrom, and E. Harlow. 1998. Substrate recruitment to cyclin-dependent kinase 2 by a multipurpose docking site on cyclin A. *Proc. Natl. Acad. Sci. USA.* 95:10453–10458.
- Shima, D.T., K. Haldar, R. Pepperkok, R. Watson, and G. Warren. 1997. Partitioning of the Golgi apparatus during mitosis in living HeLa cells. *J. Cell Biol.* 137:1211–1228.
- Stern, B., and P. Nurse. 1996. A quantitative model for the cdc2 control of S phase and mitosis in fission yeast. *Trends Genet.* 12:345–350.
- Toyoshima, F., T. Moriguchi, A. Wada, M. Fukuda, and E. Nishida. 1998. Nuclear export of cyclin B1 and its possible role in the DNA damage-induced G2 checkpoint. *EMBO (Eur. Mol. Biol. Organ.) J.* 17:2728–2735.
- Yang, J., E.S. Bardes, J.D. Moore, J. Brennan, M.A. Powers, and S. Kornbluth. 1998. Control of cyclin B1 localization through regulated binding of the nuclear export factor CRM1. *Genes Dev.* 12:2131–2143.
- Zhu, L., E. Harlow, and B.D. Dynlacht. 1995. p107 uses a p21Cip1-related domain to bind cyclin/Cdk2 and regulate interactions with E2F. *Genes Dev.* 9:1740–1752.
- Zwicker, J., F.C. Lucibello, L.A. Wolfrain, C. Gross, M. Truss, K. Engeland, and R. Muller. 1995. Cell cycle regulation of the cyclin A, cdc25C and cdc2 genes is based on a common mechanism of transcriptional repression. *EMBO (Eur. Mol. Biol. Organ.) J.* 14:4514–4522.