RanGAP1*SUMO1 is phosphorylated at the onset of mitosis and remains associated with RanBP2 upon NPC disassembly

Sowmya Swaminathan, Florian Kiendl, Roman Körner, Raffaella Lupetti, Ludger Hengst, and Frauke Melchior
Max-Planck Institute for Biochemistry, 82152 Martinsried, Germany

The RanGTPase activating protein RanGAP1 has essential functions in both nucleocytoplasmic transport and mitosis. In interphase, a significant fraction of vertebrate SUMO1-modified RanGAP1 forms a stable complex with the nucleoporin RanBP2/Nup358 at nuclear pore complexes. RanBP2 not only acts in the RanGTPase cycle but also is a SUMO1 E3 ligase. Here, we show that RanGAP1 is phosphorylated on residues T409, S428, and S442. Phosphorylation occurs before nuclear envelope breakdown and is maintained throughout mitosis. Nocodazole arrest leads to quantitative phosphorylation. The M-phase kinase cyclin B/Cdk1 phosphorylates RanGAP1 efficiently in vitro, and T409 phosphorylation correlates with nuclear accumulation of cyclin B1 in vivo. We find that phosphorylated RanGAP1 remains associated with RanBP2/Nup358 and the SUMO E2–conjugating enzyme Ubc9 in mitosis, hence mitotic phosphorylation may have functional consequences for the RanGTPase cycle and/or for RanBP2-dependent sumoylation.

Introduction

The GTPase Ran serves essential roles in nucleocytoplasmic transport, mitotic spindle formation, checkpoint control, and postmitotic nuclear envelope reassembly (Arnaoutov and Dasso, 2003; Quimby and Dasso, 2003; Walther et al., 2003). These functions are accomplished in part by the asymmetric localization of the RanGTPase activating protein RanGAP1 and the guanine-nucleotide exchange factor RCC1. RCC1 is dynamically chromatin associated in interphase and mitosis (Li et al., 2003). RanGAP1 is cytoplasmic in interphase cells, with a large fraction stably associated with the nucleoporin RanBP2/Nup358 at the cytoplasmic face of the nuclear pore complex (NPC). This interaction requires modification of RanGAP1 with the ubiquitin-related protein SUMO1 (Matunis et al., 1996; Mahajan et al., 1997). Interestingly, RanBP2 serves both as a docking factor in transport and as an E3 ligase for sumoylation (Pichler et al., 2002). In mitosis, RanGAP1 and RanBP2 are diffusely distributed throughout the cell, albeit a small fraction of both proteins is also observed at the mitotic spindle and at kinetochores (Matunis et al., 1996; Joseph et al., 2002).

To accommodate Ran’s different functions in interphase and mitosis, components of the RanGTPase cycle are likely to be cell cycle regulated. A first example is the RanGTP interacting protein RanBP1, whose levels increase from S-phase to metaphase and decline during late telophase (Guarguaglini et al., 2000). RanBP2 is hyperphosphorylated during mitosis, but the consequences of this are unknown (Favreau et al., 1996). Here, we show that RanGAP1 is subject to mitotic phosphorylation at three closely spaced residues in its COOH-terminal domain. Phosphorylation occurs at the NPC before nuclear envelope breakdown, but does not disrupt the RanGAP1*SUMO1–RanBP2–Ubc9 interaction. RanGAP1 phosphorylation may potentially alter RanGAP1’s catalytic activity or RanBP2-mediated sumoylation in vivo.

Results and discussion

RanGAP1 is phosphorylated during mitosis

To investigate whether or not RanGAP1 undergoes cell cycle–dependent changes, we synchronized HeLa cells in early S-phase and took samples as cells progressed through the cell cycle. Immunoblotting revealed that RanGAP1...
abundance and sumoylation level remained unchanged (Fig. 1 A). However, two species of SUMO1-modified RanGAP1 with lower mobility (Fig. 1 A, bands a and b) were first visible at a time coincident with the onset of mitosis (9 h after release). Maximal levels of these species were observed at 10 h after release, at which time the mitotic index was highest (34%). Cyclin A and B1 protein levels had already declined, indicating that the majority of cells had progressed through the metaphase–anaphase transition at this point. Nocodazole arrest led to quantitative conversion of sumoylated RanGAP1 into the species designated band a (Fig. 1 A). Phosphatase treatment demonstrated that bands a and b represent phosphorylated RanGAP1 (unpublished data).

Identification of three phosphorylation sites in RanGAP1

Mass spectrometry was used to identify the phosphorylated residues in RanGAP1 (Fig. 2). For this process, species represented by bands a, b, and c were enriched by immunoprecipitation from nocodazole and interphase lysates (Fig. 2 A). Bands a and c were obtained from digitonin-lysates, band b was obtained from hypotonic swelling lysates (for unknown reasons, band a was rapidly converted to band b during hypotonic swelling). Coomassie-stained bands a, b, and c were subjected to in-gel digestion by trypsin and analyzed by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (Fig. 2 B). A peptide (amino acids 407–445 in human RanGAP1) containing up to three phosphates was identified; only the unphosphorylated species was obtained from band c, up to two phosphates were detected in the peptide from band b, and two or three phosphates were present in the peptide from band a. From this analysis, we concluded that the two RanGAP1 phosphoforms a and b were phosphorylated at three and two residues, respectively. The two phosphorylated residues in band b were unequivocally identified as Ser 428 and Ser 442 through sequencing of the doubly phosphorylated peptide 414–445 by electrospray ionization ion trap mass spectrometry (Fig. 2 C).

Identification of the three sites in band a failed with this method. We considered it likely that band a was also phosphorylated at Ser 428 and Ser 442. A good candidate for the third residue was Thr 409, as it is part of a consensus motif for Cdns (TPSRK). To test if Thr 409 was phosphorylated in vivo, and to confirm phosphorylation at the other sites, we generated three phosphospecific antibodies directed toward p-T409, p-S428, and p-S442. These antibodies were used to probe RanGAP1 immunoprecipitated from interphase and nocodazole extracts (Fig. 2 D). Indeed, mitotic phosphorylation of RanGAP1 occurs at T409, S428 and S442. Band a is phosphorylated at all three residues, whereas band b is only phosphorylated at S428 and S442.

Phosphorylation of RanGAP1 occurs before nuclear envelope breakdown

Next, we tested the phosphospecific antibodies in immunofluorescence. α-p-T409 strongly decorates cells from prophase to telophase. Fig. 3 A shows representative images for different mitotic stages. In metaphase and anaphase, strong cytoplasmic staining and some enhancement at the mitotic spindle was visible, consistent with previous reports on RanGAP1 localization in mitosis (Matunis et al., 1996; Joseph et al., 2002). α-P-S428 and α-P-S442 antibodies are weaker and appear to give rise to more background, but they also clearly decorate mitotic cells (Fig. 3 B). Interestingly, relative intensities of signals between prophase and telophase varied for the three antibodies, suggesting differential timing of phosphorylation or dephosphorylation. To address this issue, we immunoprecipitated RanGAP1 from cells at different time points after thymidine release and detected RanGAP1 by immunoblotting with the phosphospecific antibodies (Fig. 3 C). At 9 h after release, band a is already...
present, whereas little singly phosphorylated S442 (comi-
grating with unphosphorylated RanGAP1) and no singly
phosphorylated T409 or S428 is detectable. This finding
suggests that RanGAP1 is initially phosphorylated at all
three sites. In contrast, dephosphorylation appears sequen-
tial, as p-T409 clearly disappears before the other two phos-
phorylated sites. Together, we find that RanGAP1 is phos-
phorylated at the onset of mitosis before nuclear envelope
breakdown. Differential timing of dephosphorylation of the
three sites in RanGAP1 results in at least three distinct mi-
mitotic RanGAP1 species: a triply phosphorylated species, and
two species phosphorylated at both S428 and S442 or only
at S442.

**Cdk phosphorylate RanGAP1**

Threonine 409 and serine 442 in human RanGAP1
(T411 and S444 in murine RanGAP1; Fig. 2 E) lie within
consensus motifs for Cdds (T/S-P-X-K/R; Kreegipuu et
al., 1999). Therefore, we tested whether or not Cdds
present in nocodazole extracts contribute to RanGAP1
phosphorylation. In vitro sumoylated RanGAP1 was incu-
bated with nocodazole extracts in the presence or absence
of the Cdk-specific inhibitor p27 (Hengst and Reed,
1998). Indeed, incubation with p27 prevented the appear-
ance of a higher molecular weight form (Fig. 4 A), indicat-
ing that Cdk activity was indeed required for Ran-
GAP1 phosphorylation. Next, we demonstrated efficient
in vitro phosphorylation of RanGAP1 with two recombi-
nant Cdds, cyclin A/Cdk2 and cyclin B/Cdk1(Fig. 4 B).
Mutagenesis of Thr 411, but not of Ser 444, severely re-
duced phosphorylation by either kinase, and phosphory-
lation was completely abolished in the double mutant
T411D, S444D (Fig. 4 B). These data demonstrate that
recombinant cyclin A/Cdk2 and cyclin B/Cdk1 phosphor-
ylate mouse RanGAP1 in vitro, with a strong preference
for Thr 411. Considering the timing and location of
RanGAP1 phosphorylation, Cdk1, but not the G1/S ki-
nase Cdk2, is a likely candidate in vivo. Three major
Cdk1 kinases contribute to mitosis, cyclin A/Cdk1, cyclin
B1/Cdk1, and cyclin B2/Cdk1. Of these, cyclin B1/Cdk1
is the most obvious candidate. Cyclin B1, which is already
expressed in the G2 phase of the cell cycle, forms a com-
plex with inactive Cdk1 and is localized in the cytoplasm.
At the onset of mitosis, cyclin B1/Cdk1 rapidly accumu-
lates in the nucleus (Yang and Kornbluth, 1999; Takizawa
and Morgan, 2000; Toyoshima-Morimoto et al., 2001).
Double labeling with α cyclin B1 and α p-T409 antibod-
ies (Fig. 4 C) demonstrated that strong nuclear envelope
labeling was first seen in prophase cells with intranuclear cyclin B1 and condensed chromatin (Fig. 4 C). Collectively, these findings make cyclin B1/Cdk1 the best candidate for phosphorylating T409.

RanGAP1 phosphorylation does not disrupt interactions with RanBP2 and Ubc9

Mitotic phosphorylation by cyclin B/Cdk1 has been suggested to facilitate disassembly and, potentially, reassembly...
of NPCs (Macaulay et al., 1995; Favreau et al., 1996). RanGAP1 phosphorylation at the G2–M transition takes place at residues directly adjacent to and within the domain required for interaction with RanBP2 (amino acids 420–470 in mouse RanGAP1; Matunis et al., 1998). Therefore, it was possible that phosphorylation disrupts this interaction as part of the NPC disassembly process. However, as shown in Fig. 5 A (top), phosphorylated RanGAP1 coimmunoprecipitated with RanBP2 from RIPA buffer extracts, indicating that the RanGAP1*SUMO1–RanBP2 complex is maintained stably in mitosis. It has previously been shown that a small fraction of the SUMO E2 enzyme Ubc9 is associated with the RanGAP1*SUMO1–RanBP2 complex at the NPC in interphase (Zhang et al., 2002). As shown in Fig. 5 A, Ubc9 also coimmunoprecipitates with RanGAP1 and RanBP2 from mitotic extracts (bottom).

Possible functions of RanGAP1 phosphorylation

We find that sumoylated RanGAP1 remains associated with RanBP2 and Ubc9 in mitosis, irrespective of its phosphorylation state. This complex regulates two different processes, the RanGTPase cycle and sumoylation of specific targets (Fig. 5 B). Therefore, two distinct functions for RanGAP1 phosphorylation can be envisaged. RanGAP1 phosphorylation may modulate RanGAP1’s catalytic activity. This modulation would have to involve recruitment of an unknown binding partner, as a direct effect of phosphorylation on the catalytic activity was not detected (Fig. S1, available at http://www.jcb.org/cgi/content/full/jcb.200309126/DC1). Alternatively, phosphorylated RanGAP1 may recruit specific SUMO target proteins to RanBP2’s catalytic domain. Interestingly, RanBP2 has recently been found to be essential for kinetochore function in mitosis (Salina et al., 2003). Although it is not known if RanBP2 targets RanGAP1 to kinetochores (Joseph et al., 2002; Arnautov and Dasso, 2003), or if the E3 ligase activity is required at this site, sumoylation does have an important role in chromosome segregation (for review see Seeler and Dejean, 2003). Identification of binding partners for the mitotic RanGAP1*SUMO1–RanBP2 complex will aid in distinguishing between these predicted functions.

Materials and methods

Plasmids and recombinant proteins

The expression plasmid for mouse RanGAP1 was as described by Mahajan et al. (1997). Mutants of RanGAP1 were engineered by site-directed mutagenesis. Purification of recombinant RanGAP1 followed published procedures (Mahajan et al., 1997). In vitro sumoylation of RanGAP1 with Aos1/Uba2 and Ubc9 was as described by Pichler et al. (2002). Bacterially expressed p27 and cyclin A/Cdk2 expressed in baculovirus were prepared as described previously (Hengst et al., 1998; Hengst and Reed, 1996). Cyclin B/Cdk1 was purchased from Calbiochem.

Antibodies

Goat α-RanGAP1, goat α-Ubc9, rabbit α-RanBP2 (Pichler et al., 2002), and rabbit α-cyclin A antibodies were described previously (Hengst and Reed, 1996). For phosphospecific antibodies, the following peptides were coupled to ovalbumin: T409, CEKSAppTSRKI; S428, CPVSppSPPPAD; and S442, CAFpSPEKLLR. Initial injection into goats was as an emulsion with Titermax Gold (Sigma-Aldrich), booster injections were with Freund’s incomplete adjuvant. Antibodies were affinity purified on phosphopeptides coupled to EAH-Sepharose 68 (Amersham Biosciences), eluted with 0.2 M acetic acid, pH 2.7, and 0.5 M NaCl, and dialyzed against PBS. Before use, antibodies were presorbed against immobilized nonphosphorylated peptides. Mouse α-cyclin B and rabbit α-GFP antibodies were obtained from Santa Cruz Biotechnology, Inc. Alexa 488 donkey α goat was purchased from Molecular Probes, other secondary antibodies were purchased from Jackson ImmunoResearch Laboratories.

Immunoblotting and fluorescence microscopy

Detection of antigens on nitrocellulose was performed with affinity-purified goat α-pT409 (0.5 µg/mL), α-pS428 (0.4 µg/mL), α-pS442 (0.7 µg/mL), or α-RanGAP1 (0.25 µg/mL) in 5% milk powder (or 0.2% gelatine) in PBS, 0.2% Tween 20 for 1 h at RT. Detection was performed by chemiluminescence. For indirect immunofluorescence, adherent HeLa cells grown on coverslips were either fixed for 10 min with 2% PFA in PBS, 1 mM MgCl₂, and permeabilized for 5 min with 0.2% Triton X-100 in PBS, 1 mM MgCl₂, or permeabilized and fixed for 10 min in 4% PFA, 0.2% Triton X-100, 20 mM PIPES, 1 mM MgCl₂, and 10 mM EGTA (Kapoor et al., 2000b). Antibodies in 2% BSA, PBS/MgCl₂ were used at 0.25 µg/mL.
The Journal of Cell Biology  Volume 164, Number 7, 2004

Immunoprecipitations
Immunoprecipitations were from SDS-lysates, digoxigenin cytosol, cytosol generated by hypotonc swelling, or from RIPA extracts from cycling and nocodazole-arrested HeLa cells. For SDS-lysate, HeLa suspension cells were lysed by boiling in 1% SDS and diluted 10-fold with RIPA buffer-SDS (50 mM Tris-HCl, pH 8, 150 mM NaCl, 1% NP-40, 0.5% deoxycholate, 1 mM DTT, and 1 µg/ml pepstatin, aprotinin, and leupeptin). Hypotonc swelling extracts were generated as described previously (Melchior, 1998), and buffer was changed to TB buffer with protease inhibitors and phos- phatase inhibitor cocktail I (Sigma-Aldrich) using PD10 columns (Amer- sham Biosciences). For digoxigenin cytosol, HeLa cells were lysed in TB con- taining protease inhibitors and phosphatase inhibitor cocktail I and 0.005% digoxigenin (Calbiochem). For RIPA extracts, HeLa suspension cells were lysed by sonication in 4 vol RIPA buffer with protease inhibitors, phosphatase inhibitor cocktail I, and 10 mM iodoacetamide. All cell lys- ates were clarified at 100,000 g for 45 min before use in IP. Affinity-puri- fied antibodies or control IgGs cross-linked at 2 mg/ml to Ultralink Immuno- grade; Promega) using essentially the protocol of Shevchenko et al. Coomassie-stained protein bands were in-gel digested by trypsin (sequenc- ing grade; Promega) and analyzed on an Applied Biosystems 4700A (Applied Biosystems). MALDI-TOF mass spectra were acquired on a Reflex III instrument (Bruker Daltonik) in positive ion reflector mode. As a reference, myoglobin (sheep) (Promega) was used.

Cell cycle analysis of RanGAP1 phosphorylation
A standard double thymidine block release protocol was used to obtain a synchronized population of suspension HeLa cells (Bonifacino et al., 1999). At indicated times, cells were harvested by centrifugation, aliquots flash frozen, and stored at −80°C. Aliquots were used for analysis by immunoblotting upon lysis in Laemmli buffer or for immunoprecipitation upon SDS-lysis. Progression through the cell cycle was monitored by FACS® analysis after cell fixation in 70% ethanol and staining with propidium io- dide (Bonifacino et al., 1999). To determine the mitotic index, cells were fixed in 70% ethanol, stained using a final concentration of 4 µg/ml Hoechst 33342 (Molecular Probes), mounted with Glue mounting me- dium (EnerGene), and observed using a microscope (model Axioskop II; Carl Zeiss Microimaging, Inc.).

In vitro RanGAP1 phosphorylation
Phosphorylation of 2 µg RanGAP1 with recombinant kinases was in 20 mM Tris-HCl, pH 7.5, 10 mM MgCl2, 50 µM ATP, and 10 µCi [γ-32P]ATP at 30°C for 30 min. Cyclin B/Cdk1 (Calbiochem) and cyclin A/Cdk2 were used at 2 µg/ml or 5 ng, respectively. Analysis was performed by SDS-PAGE and autoradiography. Mitotic extracts for RanGAP1 phosphorylation were prepared from 100 ml of nocodazole-arrested HeLa cells by freeze-thaw lysis in 1.5 ml TB buffer supplemented with phosphatase inhibitor cocktail I. 100 ng of SUMO1-modified RanGAP1 was incubated in 5 µl of extracts and 1 mM of ATP at 30°C for 2 h. Reconstituent p27 at concentrations of 1 µg or 5 µg was used to pretreat mitotic cell extracts on ice for 45 min. Re- actions were analyzed by immunoblotting with a RanGAP1 antibodies.

Mass spectrometry
Coomassie-stained protein bands were in-gel digested by trypsin (sequenc- ing grade; Promega) using essentially the protocol of Shevchenko et al. (1996) and desalted using home-made miniaturized reversed-phase col- umns (Gobom et al., 1999). MALDI-TOF mass spectra were acquired on a Reflex III instrument (Bruker Daltonik) in positive ion reflector mode. As a matrix, 2,5 dihydroxybenzoic acid (Bruker Daltonik) was used. For peptide sequence analysis by electrospray tandem mass spectrometry, samples were filled into nano electrospray needles (Protana) and analyzed on an Applied Biosystems 4700A (Applied Biosystems). MALDI-TOF mass spectra were acquired on a Reflex III instrument (Bruker Daltonik) in positive ion reflector mode. As a reference, myoglobin (sheep) (Promega) was used.

Online supplemental material
The supplemental material is available at http://www.jcb.org/cgi/content/full/ jcb.200309126/DC1.

We are grateful for many stimulating discussions with Dr. Andrea Pichler and other members of the laboratory. Dr. Frank Freudenmann is ack-nowledged for peptide synthesis and Dr. Heinz Brandstetter for immu- nization services.

This work was funded by the Bundesministerium für Bildung und For- schung (grant BioFUTURE 0311869), an Alexander von Humboldt fellow- ship (to S. Swaminathan), and the Max-Planck Institute for Biochemistry.

Submitted: 22 September 2003
Accepted: 18 February 2004

References

Ammourout, A., and M. Dasso. 2003. The Ran GTPase regulates kinetochore func- tion. Dev. Cell. 5:99–111.
Bonifacino, J.S., M. Dasso, J. Lippincott-Schwartz, J.B. Harford, and K.M. Ya- mada. 1999. Current Protocols in Cell Biology. John Wiley and Sons, New York. Also available at http://www.mrw2.interscience.wiley.com/cpronline.
Favreau, C., H.J. Worman, R.W. Wozniak, T. Frappier, and J.C. Courvalin. 1996. Cyclin-dependent phosphorylation of nuclear proteins and nuclear pore membrane protein Gp210. Biochemistry. 35:8035–8044.
Gobom, J., E. Nordhoff, E. Mirgorodskaya, R. Ekmann, and P. Roestorff. 1999. Sample purification and preparation technique based on nano-scale re- versed-phase columns for the sensitive analysis of complex peptide mixtures by matrix-assisted laser desorption/ionization mass spectrometry. J. Mass Spectrom. 34:105–116.
Guarguaglini, G., L. Renzi, F. D’Oratorio, B. Di Fiore, M. Casenghi, E. Candiari, and P. Lavia. 2000. Regulated Ran-binding protein 1 activity is required for organization and function of the mitotic spindle in mammalian cells in vivo. Cell Growth Differ. 11:455–465.
Hengst, L., and S.I. Reed. 1996. Translational control of p27Kip1 accumulation during the cell cycle. Science. 271:1861–1864.
Hengst, L., and S.I. Reed. 1998. Inhibitors of the Cip/Kip family. Curr. Top. Mi- crobiol. Immunol. 227:25–41.
Hengst, L., U. Gopfert, H.A. Lashuel, and S.I. Reed. 1998. Complete inhibition of Cdk/cyclin by one molecule of p21(Cip1). Gene Dev. 12:3882–3888.
Joseph, J., S.H. Tan, T.S. Karpova, J.G. McNally, and M. Dasso. 2002. SUMO-1 targets RanGAP1 to kinetochore and mitotic spindles. J. Cell Biol. 156: 595–602.
Kapoor, T.M., T.U. Mayer, M.L. Coughlin, and T.J. Mitchison. 2000. Probing spindle assembly mechanisms with monastrol, a small molecule inhibitor of the mitotic kinase, Eg5. J. Cell Biol. 150:975–988.
Kreegipuu, A., N. Blom, and S. Brunnak. 1999. PhosphoBase, a database of phospho- tyrosylation sites: release 2.0. Nucleic Acids Res. 27:237–239.
Li, H.Y., D. Wirtz, and Y. Zheng. 2003. A mechanism of coupling RCC1 mobility to RanGTP production on the chromatin in vivo. J. Cell Biol. 160:635–644.
Macaw, C., E. Meier, and D.J. Forbes. 1995. Differential mitotic phosphoryla- tion of proteins of the nuclear pore complex. J. Biol. Chem. 270:254–262.
Maahajan, R., C. Delphin, T. Guan, L. Gerace, and F. Melchior. 1997. A small ubiquitin-related polypeptide involved in targeting RanGAP1 to nuclear pore complex protein RanBP2. Cell. 88:97–107.
Marutis, M.J., E. Courtaux, and G. Blobel. 1996. A novel ubiquitin-like modifica- tion modulates the partitioning of the Ran-GTPase-activating protein RanGAP1 between the cytosol and the nuclear pore complex. J. Cell Biol. 135:1457–1470.
Marutis, M.J., J. Wu, and G. Blobel. 1998. SUMO-1 modification and its role in targeting the Ran GTPase-activating protein, RanGAP1, to the nuclear pore complex. J. Cell Biol. 140:499–509.
Melchior, F. 1998. Nuclear protein import in a permeabilized cell assay. Methods Mol. Biol. 88:265–273.
Pichler, A., A. Gast, J.S. Seeler, A. Dejean, and F. Melchior. 2002. The nucle- ooporin RanBP2 has SUMO1 E3 ligase activity. Cell. 108:109–120.
Quimby, B.B., and M. Dasso. 2003. The small GTPase Ran: interpreting the signs. Curr. Opin. Cell Biol. 15:338–344.
Salina, D., P. Enarson, J.B. Ramtrier, and B. Burke. 2003. Nup538 integrates nuclear envelope breakdown with kinetochore assembly. J. Cell Biol. 162:991–1001.
Seeler, J.S., and A. Dejean. 2003. Nuclear and unclear functions of SUMO. Nat. Rev. Mol. Cell Biol. 4:690–699.
Shevchenko, A., M. Wilm, O. Vorm, and M. Mann. 1996. Mass spectrometric se- quencing of proteins from silver-stained polyacrylamide gels. Anal. Chem. 68:850–858.
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Takizawa, C.G., and D.O. Morgan. 2000. Control of mitosis by changes in the subcellular location of cyclin-B1-Cdk1 and Cdc25C. Curr. Opin. Cell Biol. 12:658–665.

Toyoshima-Morimoto, F., E. Taniguchi, N. Shinya, A. Iwamatsu, and E. Nishida. 2001. Polo-like kinase 1 phosphorylates cyclin B1 and targets it to the nucleus during prophase. Nature. 410:215–220.

Walther, T.C., P. Askjaer, M. Gentzel, A. Habermann, G. Griffiths, M. Wilm, I.W. Mattaj, and M. Hetzer. 2003. RanGTP mediates nuclear pore complex assembly. Nature. 424:689–694.

Yang, J., and S. Kornbluth. 1999. All aboard the cyclin train: subcellular trafficking of cyclins and their CDK partners. Trends Cell Biol. 9:207–210.

Zhang, H., H. Saitoh, and M.J. Matunis. 2002. Enzymes of the SUMO modification pathway localize to filaments of the nuclear pore complex. Mol. Cell. Biol. 22:6498–6508.