Interaction of Chromatin-associated Plk1 and Mcm7*

Lyuben Tsvetkov and David F. Stern‡

From the Department of Pathology, School of Medicine, Yale University, New Haven, Connecticut 06511

Plk1 is a multifunctional protein kinase involved in regulation of mitotic entry, chromosome segregation, centrosome maturation, and mitotic exit. Plk1 is a target of DNA damage checkpoints and aids resumption of the cell cycle during recovery from G2 arrest. The polo-box domain (PBD) of Plk1 interacts with phosphoproteins and localizes Plk1 to some mitotic structures. In a search for proteins that interact with the PBD of Plk1, we identified two of the minichromosome maintenance (MCM) proteins, Mcm2 and Mcm7. Co-immunoprecipitation and immunoblot analysis showed an interaction between full-length Plk1 and all other members of the MCM2–7 protein complex. Endogenous Plk1 co-immunoprecipitates with basal forms of Mcm7 as well as with slower migrating forms of Mcm7, induced in response to DNA damage. The strongest interaction between endogenous Plk1 and Mcm7 was detected in a soluble chromatin fraction. These findings suggest a new function for Plk1 in coordination of DNA replication and mitotic events.

Normal progression of mammalian cells through the cell cycle requires precise integration of positive and negative cell cycle regulators. This function is fulfilled by biochemical networks, called cell cycle checkpoints, that prevent the initiation of the next cell cycle event before correct execution of the previous one. One of the critical circuits for cell cycle control activates Cdc2/cyclin B kinase, called maturation-promoting factor (MPF), at the onset of mitosis. Wee1 and Myt1 kinases keep MPF inactive by inhibitory phosphorylation of Cdc2, whereas phosphatases from Cdc25 family remove these phosphates and activate MPF. Polo-like kinase 1 (Plk1) is one of the protein kinases that activate Cdc25C.

Plk1 is a positive cell cycle regulator that is a member of the Polo-like kinase (PLK) family. Plk1 is expressed in S, G2, and M phases of the cell cycle, and becomes activated by phosphorylation at the G2/M boundary. Plk1 regulates a cleavage-independent mechanism for dissociation of cohesin from chromosomes, which is important for the separation of sister chromatids (1). Plk1 assists in MPF activation through phosphorylation-dependent import of cyclin B1 and activation of Cdc25C, an activator of Cdc2. After metaphase, Plk1 enhances ubiquitin-dependent degradation of cyclin B1 and inactivation of MPF by activation of anaphase-promoting complex. Results of experiments with a dominant negative mutant of Plk1 suggest a possible role for Plk1 in cytokinesis (2). The centrosome cycle requires Plk1, because microinjection of α-Plk1 antibodies prevents centrosome maturation (3).

DNA damage response pathways, including ATM and ATR, inhibit Plk1 after DNA damage (4, 5). Interactions of Plk1 with Chk2, Claspin, and Brca1 suggest that there are additional roles of Plk1 in DNA structure checkpoints (6–8). Plk1 is also involved in restarting the cell cycle after DNA damage-induced checkpoint arrest, through down-regulation of Wee1 (9).

PLKs have one or two conserved elements, called polo boxes, which form carboxyl-terminal phosphopeptide-binding domains (PBDs) (10). The PBD is probably involved in regulation of Plk1, because binding of the PBD to a phosphopeptide elevates Plk1 kinase activity (11). PBDs are important for localization of PLKs to mitotic structures (12) and PLK interactions with other proteins, including Cdc25C, MKlp2, and Chk2 (10, 13). Identification of additional Plk1 PBD-interacting proteins may lead to discovery of new functions of Plk1.

Six of the minichromosome maintenance (MCM) proteins, Mcm2-Mcm7, form complexes that participate in initiation and elongation steps of DNA replication (14). They share a conserved 200-amino acid nucleotide-binding region and form different subcomplexes (dimers, trimers, and a hexamer) (15). Mcm4-Mcm6-Mcm7 trimers and hexamers (Mcm2-Mcm7) have ATPase and DNA helicase activities in vitro (14). MCM proteins are associated with chromatin in late telophase and at the beginning of the G1 phase of the cell cycle (16). Interaction of MCMs with chromatin depends on proteins of the origin recognition complex (ORC), Cdc6 and Cdt1 (17). These proteins form a pre-replication complex at origins of DNA replication. Pre-replication complexes are activated by cyclinE/Cdk2, cyclinA/Cdk2, and Dbf4/Cdc7 protein kinases at the G1/S phase transition (14). During S phase, Mcm proteins are released from origins of replication after initiation of DNA replication and move with replication forks where they are thought to function as a DNA helicase. Mechanisms that assure the replication of DNA only once per cycle release Mcm proteins from chromatin after firing of the origins of replication and prevent the reloading of Mcm proteins on chromatin until telophase. Mcm7 participates in processes other than DNA replication, interaction with the MYCN transcription factor (18) and regulation of its own transcription, together with Mcm1 (19). Mcm2 and Mcm3 are substrates for checkpoint transducers ATM and ATR, and Mcm7 interacts with the ATR partner protein ATR-interacting protein. Knockdown of Mcm7 interferes with S phase checkpoint function (20).

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‡ To whom correspondence should be addressed: Dept. of Pathology, School of Medicine, Yale University, 310 Cedar St., BML 342, New Haven, CT 06511. Tel.: 203-785-4832; Fax: 203-785-7467; E-mail: DFStern@yale.edu.

1 The abbreviations used are: MPF, maturation-promoting factor; α-, anti-, ATM, ataxia telangiectasia-mutated; ATR, ATM- and Rad3-related; PLK, Polo-like kinase; GST, glutathione S-transferase; HA, hemagglutinin; IB, immunoblot; MALDI-MS, matrix-assisted laser desorption ionization mass spectrometry; MCM, minichromosome maintenance protein; ORC, origin recognition complex; PBD, Polo-box domain; IP, immunoprecipitation; mAb, monoclonal antibody; PIPES, 1,4-piperazinediethanesulfonic acid.

2 L. Tsvetkov and D. F. Stern, unpublished data.
In this study, we identify members of the MCM protein complex as Plk1-interacting proteins. Interaction of Plk1 with Mcm2 and Mcm7 requires the Plk1 PBD. The interaction between Plk1 and Mcm7 is regulated in response to DNA damage. Chromatin-associated Plk1 and Mcm7 interact more strongly than their cytosolic forms. These results provide evidence for the existence of a link between the DNA replication apparatus and mitotic regulation.

EXPERIMENTAL PROCEDURES

Plasmids, Cloning, Expression, and Purification—pcDNA3–3xFLAG-Plk1, pcDNA3–3xFLAG-Plk1(1–330), and pcDNA3–3xFLAG-Plk1(330-CT) were previously described (6). Plk1 was cloned into the pGEX4T3 vector to produce pGEX4T3-GST-Plk1. Expression and purification of GST and GST-Plk1 proteins were carried out as described previously (21).

Cell Cultures, Treatments, and Transfection—HEK293 cells were grown in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum in the presence of antibiotics in a humidified incubator at 37 °C. Cells were treated with nocodazole (250 ng/ml, Sigma) for 16 h, hydroxyurea (1 mM, Sigma) for 16 h, or Adriamycin (0.5 or 2 μM, Sigma) for 2 h. Cells were transfected with FuGENE 6 (Roche Applied Science), 5 μg of plasmid DNA per 10-cm culture dish, and analyzed after 40 h.

Antibodies, Immunoprecipitation, and Immunoblot—Cells were washed in phosphate-buffered saline and solubilized in lysis buffer (50 mM Tris-Cl, pH 7.5, 0.5% Nonidet P-40, 120 mM NaCl) containing a protease inhibitor mixture (Roche Applied Science). For immunoprecipitation (IP), 500 μg of cell lysate was incubated at 4 °C for 16 h with anti-α-Plk1 mAb mixture (Zymed Laboratories) and α-GST M2 mAb (Sigma), and 20 μl of protein G plus/protein A-agarose (Oncogene Research Products), or α-FLAG affinity-agarose gel (Sigma).

Immunocomplexes and lysates were analyzed by SDS-PAGE, and immunoblotting was performed according to standard techniques with horseradish peroxidase-conjugated mouse α-FLAG-M2 mAb (Sigma), mouse α-Plk1 (F8) mAb, mouse α-MCM7 (141.2) mAb (Santa Cruz Biotechnologies), rabbit polyclonal α-Mcm3 AB (Calbiochem), mouse α-Orcl2 mAb and mouse α-Grb2 mAb (BD Biosciences) and horseradish peroxidase-conjugated secondary antibodies (Pierce). Immunoblotted proteins were detected using ECLTM (Amersham Biosciences) and SuperSignal West Femto secondary antibodies (Pierce). Immunoprecipitated proteins were separated by SDS-PAGE, and proteins were visualized by staining with Coomassie Blue G-250.

Cell Fractionation—Soluble chromatin fractions were prepared according to the procedure described by Fujita et al. (22). Briefly, 293-T cells were lysed on ice with CSK buffer (10 mM PIPES, pH 6.8, 100 mM NaCl, 1 mM MgCl₂, 1 mM EDTA, 1 mM dithiothreitol, and 300 mM sucrose) containing 0.1% Triton X-100, and centrifuged at 1,000 x g for 5 min. The supernatants were clarified by centrifugation at 15,000 x g for 5 min to prepare Triton X-100-extractable fractions. The pellets, containing nuclei, were washed, resuspended in CSK buffer containing 1 mM ATP and digested with 10 units/μl DNase I at 25 °C for 30 min to prepare DNase I-released fractions.

RESULTS AND DISCUSSION

To identify Plk1-interacting proteins, α-FLAG antibody was used to immunoprecipitate lysates from HEK293-T cells transiently expressing a FLAG-tagged portion of Plk1, amino acids 330 to the carboxyl terminus, that constitutes the PBD (10). Immunoprecipitated proteins were separated by SDS-PAGE and stained with Coomassie Blue G-250 (Fig. 1). Two protein bands with molecular masses of 125 and 90 kDa that were specific for immunoprecipitates from cells expressing FLAG-Plk1-(330-CT) were excised from the gel and analyzed by MALDI-MS. They were identified as minichromosome maintenance proteins 2 (Mcm2) and 7 (Mcm7).

To confirm the interaction between FLAG-Plk1 and Mcm7, we used an α-Mcm7 antibody to blot α-FLAG immunoprecipitates from lysates from cells transiently expressing FLAG-Plk1. Mcm7 was detected only in α-FLAG immunoprecipitates but not in control IgG immunoprecipitates (Fig. 2A, upper panel, lanes 2 and 3) of α-FLAG immunoprecipitates from vector transfected cells (Fig. 2A, upper panel, lane 4). To determine the involvement of Plk1 polo-box and the kinase domains in Plk1 interaction with Mcm7, α-FLAG immunoprecipitates from lysates from cells transfected with a vector or cells transiently expressing FLAG-Plk1-(330-CT), and FLAG-Plk1-(330-CT), were immunoblotted with an α-Mcm7 antibody (Fig. 2B, upper panel). Mcm7 was present in higher amounts in immunoprecipitates from cells expressing FLAG-Plk1 and FLAG-Plk1-(330-CT) (Fig. 2B, lanes 3 and 5), in lesser amounts in immunoprecipitates from cells expressing FLAG-Plk1-(1–330) (lane 4), and was absent in immunoprecipitates from control-transfected cells (lane 2).
another experimental approach, GST-Plk1 and GST proteins were immobilized on glutathione beads and incubated with lysates from HEK293-T cells. Mcm7 was pulled down by GST-Plk1 (Fig. 2D, lane 2), but not by GST (lane 1).

Six Mcm proteins, Mcm2–7, can associate in a single complex and are also assorted among various sub-complexes. We determined whether other members of the complex, in addition to Mcm2 and Mcm7, also interact with Plk1. α-FLAG immunoprecipitates from cells expressing FLAG-Plk1, FLAG-Plk1-(1–330), and FLAG-Plk1-(330-CT) were probed with an α-Mcm3 antibody. Mcm3 was only detected in immunoprecipitates from cells expressing full-length FLAG-Plk1 (Fig. 3A, lane 2). This differs from the binding requirements for Mcm7, which also binds well to the 330-CT fragment containing the PBD (Fig. 2B, lane 5).

To evaluate the presence of the other Mcm proteins in FLAG-Plk1 immunoprecipitates, we used an α-MCM2–7 antibody (generously provided by S. Bell) that recognizes a conserved epitope on all MCM2–7 subunits (23). The antibody detected Mcm2–7 in the lysates as well as in α-FLAG-Plk1 immunoprecipitates from HEK 293T cells expressing FLAG-Plk1 (Fig. 3B, lane 4).

To find a biological context for the interaction between Plk1 and Mcm7, we determined if DNA damage affects the interaction. To introduce DNA damage, we used Adriamycin, a topoisomerase inhibitor that causes DNA breaks. This activates DNA damage checkpoints in multiple phases of the cell cycle (24). Treatment of HEK 293 cells with Adriamycin increased the amount of slower migrating forms of Mcm7 (Fig. 4A, lanes 2 and 3). Interestingly, slower migrating forms of Mcm7 were proportionately increased relative to basal Mcm7 forms in Plk1 immunoprecipitates (Fig. 4B, lanes 3 and 4). A protein band with the same mobility was detected by the α-MCM2–7 antibody that recognizes all MCM2–7 subunits (23) in Plk1 immunoprecipitates from Adriamycin-treated cells (Fig. 4C, lane 5), but not from control untreated cells (lanes 4 and 6), and in antibody only immunoprecipitates (lane 3). Treatment of the immunoprecipitates with λ-phosphatase decreased the slower migrating forms detected with α-Mcm7 (Fig. 4B, lane 5) and α-Mcm2–7 antibodies, suggesting that these are phosphoproteins.

Mass spectrometry and IB data showed that Plk1 interacts with Mcm2 through Mcm7. However, it is not clear if Plk1 interacts with Mcm3 and other MCM proteins in a biological context. The antibody detected Mcm2–7 in the lysates as well as in α-FLAG-Plk1 immunoprecipitates from HEK 293T cells expressing FLAG-Plk1 (Fig. 3B, lane 4).

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binds each of the MCMs separately or if one or more individual MCMs mediate the interaction of the complex with Plk1. The PBD of Plk1 is required for the interaction of Plk1 with Mcm3 and Mcm7, so it is possible that this interaction is phosphorylation-dependent (Figs. 2B and 3A). However, the interaction of Mcm3 with FLAG-Plk1 requires full-length Plk1, indicating participation of the kinase and PBD domains of Plk1 in the interaction with MCMs (Fig. 3A).

Mcm proteins are localized to the cytosol and nucleus and are associated with chromatin in a cell cycle-dependent manner. Cytosolic extracts were used for the immunoprecipitation experiments in Figs. 1–4. Endogenous Plk1 and MCMs might be expected to interact at chromatin, because Mcm proteins are associated with chromatin in parts of late mitosis, G1, and S phases, and Plx1 is present at stalled replication forks in association with Claspin (8). To examine the association of Mcm7, as a representative Mcm protein, and Plk1 with chromatin, we fractionated cellular lysates into cytosolic (Triton X-100-extractable) and "soluble chromatin" fractions prepared by DNase treatment of Triton X-100-extracted nuclei (22). Plk1 and Mcm7 proteins were present in both sets of fractions (Fig. 5, lanes 2–7). Orc2, a chromatin-associated protein, was used as a cytosolic marker (Fig. 5, lower two panels). Mcm7 was co-immunoprecipitated with endogenous Plk1 from both cytosolic and DNase-released chromat fractions (Fig. 5, lanes 5 and 2). Interestingly, the α-Plk1 antibody co-immunoprecipitated more Mcm7 from the DNase-released soluble chromatin fraction than from the cytoplasmic fraction, even though both Plk1 and Mcm7 were more abundant in the cytosolic fraction (Fig. 5, lanes 2 and 5). Treatment of cells with nocodazole for 16 h synchronized them in G2/M. Under these conditions, the amount of total Mcm7 and Mcm7 in α-Plk1 immunoprecipitates from the soluble chromatin fraction was reduced. Likewise, S-phase arrest imposed by hydroxyurea treatment for 24 h did not alter the relative association between Plk1 and Mcm7 (Fig. 5, lanes 4 and 7). Hydroxyurea treatment inhibits DNA synthesis, activating the intra-S phase replication checkpoint, and activating DNA damage checkpoint pathways.

The budding yeast Polo-like kinase Cdc5 interacts with the Dbf4 subunit of Dbf4/Cdc7 kinase that activates the pre-replication complex (26). Dbf4/Cdc7 itself phosphorylates some of the Mcm proteins (27). Also, Rad53, the Chk2 ortholog in budding yeast, regulates Dbf4/Cdc7 kinase after DNA damage (28). These results, taken together with the fact that proteins from the MCM complex are also located at the replication fork, would be consistent with the stronger co-immunoprecipitation of Mcm7 with Plk1 from chromatin, relative to cytosolic fractions (Fig. 4).

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A subset of the MCM proteins are phosphorylated in S phase by Cdk2 and Dbf4/Cdc7 protein kinases, which are required for S phase entry and for activation of replication forks, and by the checkpoint phosphoinositide 3-kinase-related kinase ATR, which negatively regulates cell cycle progression after DNA damage, and which might protect stalled replication forks (20, 25). The requirement of the Plk1 PBD, a phosphopeptide-binding motif, for Plk1 binding to MCM7 and MCM3 proteins, suggests that a phosphorylation-dependent mechanism mediates the interaction (Figs. 2B and 3A). Perhaps phosphorylation of MCMs by Dbf4/Cdc7 and S phase cyclin-dependent kinases, or by ATR or ATM kinases creates binding sites for Plk1 PBD. In a Xenopus DNA replication checkpoint response to stalled replication forks, ATR phosphorylates chromatin-associated Claspin, a checkpoint mediator protein, creating a docking site through which the Polo-like kinase Plx1 interacts with Claspin (8). Plk1 bound to Claspin, phosphorylates it, and causes Claspin dissociation from chromatin (8). Such a model, together with the fact that proteins from the MCM complex are also located at the replication fork, would be consistent with the stronger co-immunoprecipitation of Mcm7 with Plk1 from chromatin, relative to cytosolic fractions (Fig. 4).
signals (29). In the context of these results, it is even more intriguing that slower migrating forms of Mcm7, induced by DNA damage after treatment with Adriamycin, co-immunoprecipitate efficiently with Plk1 (Fig. 4B, lane 4; Fig. 4C, lane 5).

Strict timely regulation of Plk1 expression and activity is important for the passage of cells through mitosis. Overexpression of a kinase-defective mutant of Plk1 (Plk1-KD), the carboxyl-terminal portion of Plk1 (Plk1-CT), and, to a lesser extent, the wild type Plk1, alters the normal cell cycle of some cell culture lines, resulting in the accumulation of cells with G2/M DNA content and disorganization of condensed chromosomes (30, 31). Furthermore, spindle checkpoint mechanisms are activated after overexpression of Plk1-KD and Plk1-CT (2). These cells later undergo mitotic catastrophe and/or apoptosis. The fact that kinase inactive mutants as well as wild type Plk1 induce this cellular phenotype, rules out the involvement of Plk1 kinase activity. One possible explanation for this phenotype is the titration of a protein or proteins crucial for the transition through mitosis. Overexpressed PBD of Plk1 binds to and inhibits endogenous Plk1, which might also cause this abnormal phenotype (32). Introduction of a polo-box peptide fused to an Antennapedia peptide in cells results in G2/M cell cycle arrest, misaligned chromosomes, multiple spindle poles, and apoptosis (33).

The interaction of Plk1-(330-CT) with Mcm proteins suggests new mechanisms for the phenotype observed after expression of Plk1-CT. Plk1-(330-CT) binding to different Mcm protein complexes might affect their functions in DNA replication, such as firing of DNA replication origins. This could produce altered complexes might affect their functions in DNA replication, such as MCM complexes involved in regulation of DNA replication. The association of Plk1 with Mcm proteins indicates possible new functions for Plk1, as well as for Mcm proteins and the possibility of a new link for coordination between DNA replication and mitosis, in the unperturbed cell cycle and in response to genotoxic stress.

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REFERENCES

1. Sumara, I., Vorlaufer, E., Stukenberg, P. T., Kelm, O., Redemann, N., Nigg, E. A., and Peters, J. M. (2002) Mol. Cell 9, 515–525
2. Seong, Y. S., Kamiyo, K., Lee, J. S., Fernandez, E., Kuriyama, R., Miki, T., and Lee, K. S. (2002) J. Biol. Chem. 277, 32282–32293
3. Lane, H. A., and Nigg, E. A. (1996) J. Cell Biol. 135, 1701–1713
4. Smits, V. A., Klompaker, R., Arnaud, L., Rijksen, G., Nigg, E. A., and Medema, R. H. (2000) Nat. Cell Biol. 2, 672–676
5. van Vught, M. A., Smits, V. A., Klompaker, R., and Medema, R. H. (2001) J. Biol. Chem. 276, 41656–41660
6. Tsvetkov, L., Xu, X., Li, J., and Stern, D. F. (2003) J. Biol. Chem. 278, 8468–8475
7. Lee, M., Daniele, M. J., and Venkitaraman, A. R. (2004) Oncogene 23, 865–872
8. Yoo, H. Y., Kumagai, A., Shevchenko, A., and Dunphy, W. G. (2004) Cell 117, 575–588
9. Van Vught, M. A., Bras, A., and Medema, R. H. (2004) Mol. Cell 15, 799–811
10. Elia, A. E., Cantley, L. C., and Yaffe, M. B. (2003) Science 299, 1228–1231
11. Elia, A. E., Retlos, P., Haire, L. F., Chao, J. W., Ivins, F. J., Hoeperk, R., Mohammad, D., Cantley, L. C., Smerdon, S. J., and Yaffe, M. B. (2003) Cell 115, 83–95
12. Lee, K. S., Grenfell, T. Z., Yarm, F. R., and Erikson, R. L. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 9301–9306
13. Neef, R., Preisinger, C., Sutcliffe, J., Kopajtich, R., Nigg, E. A., Mayer, T. U., and Barr, F. A. (2003) J. Cell Biol. 162, 863–875
14. Lei, M., and Tye, B. K. (2001) J. Cell Biol. 114, 1447–1454
15. Reznik, E. V. (1993) Nucleic Acids Res. 21, 2541–2547
16. Dimitrova, D. S., Prokhorova, T. A., Blow, J. J., Todorov, I. T., and Gilbert, D. M. (2002) J. Cell Sci. 115, 51–59
17. Tanaka, T., Knapp, D., and Nasmyth, K. (1997) Cell 90, 649–660
18. Shihet, J. M., Hidve, M. J., Plon, S. E., Burlingame, S. M., Stuart, S., Chen, S. Y., Brenner, M. K., and Nuchtern, J. G. (2002) Cancer Res. 62, 1123–1128
19. Pitch, M. J., Donato, J. J., and Tye, B. K. (2003) J. Biol. Chem. 278, 25408–25416
20. Cortez, D., Glick, G., and Elledge, S. J. (2004) Proc. Natl. Acad. Sci. U. S. A. 101, 10078–10083
21. Xu, X., Tsvetkov, L. M., and Stern, D. F. (2002) Mol. Cell. Biol. 22, 4419–4432
22. Fujita, M., Yamada, C., Tsurumi, T., Hanaoka, F., Matsuoka, K., and Inagaki, M. (1998) J. Biol. Chem. 273, 17095–17101
23. Klemm, R. D., and Bell, S. P. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 8361–8367
24. Tewey, K. M., Rowe, T. C., Yang, L., Halligan, B. D., and Liu, L. F. (1984) Science 226, 466–468
25. Forssberg, S. L. (2004) Microbiol. Mol. Biol. Rev. 68, 109–131
26. Hardy, C. P., and Pauzat, A. (1996) Mol. Cell. Biol. 16, 6775–6782
27. Lei, M., Kawasaki, Y., Young, M. R., Kihara, M., Sugino, A., and Tye, B. K. (1997) Genes Dev. 11, 3363–3374
28. Dohrmann, P. R., Oshiro, G., Tecklenburg, M., and Sclafani, R. A. (1999) Genetics 151, 965–977
29. Tao, C. C., Geisen, C., and Abraham, B. T. (2004) EMBO J. 23, 4660–4669
30. Mundt, K. H., Golsteyn, R. M., Lane, H. A., and Nigg, E. A. (1997) Biochem. Biophys. Res. Commun. 239, 377–383
31. Cogswell, J. P., Brown, C. E., Bisi, J. E., and Neill, S. D. (2000) Cell Growth & Differ. 11, 615–623
32. Jiang, Y. J., Lin, C. Y., Ma, S., and Erikson, R. L. (2002) Proc. Natl. Acad. Sci. U. S. A. 99, 1984–1989
33. Yuan, J., Kramer, A., Eckerdt, F., Kaufmann, M., and Strebhardt, K. (2002) Cancer Res. 62, 4186–4190
34. Garber, P. M., and Rine, J. (2002) Genetics 161, 521–534
35. Prasanth, S. G., Prasanth, K. V., and Stillman, B. (2002) Science 297, 1026–1031
