The Ste20-like Kinase SLK Is Required for Cell Cycle Progression through G2

Paul G. O’Reilly, Simona Wagner, Douglas J. Franks, Katia Cailliau, Edith Browaeys, Colette Dissous, and Luc A. Sabourin

We have previously shown that the Ste20-like kinase SLK is a microtubule-associated protein that can regulate actin reorganization during cell adhesion and spreading (Wagner, S., Flood, T. A., O’Reilly, P., Hume, K., and Sabourin, L. A. (2002) J. Biol. Chem. 277, 37685–37692). Because of its association with the microtubule network, we investigated whether SLK plays a role in cell cycle progression, a process that requires microtubule dynamics during mitosis. Consistent with microtubule association in exponentially growing cells, our results showed that SLK co-localizes with the mitotic spindle in cells undergoing mitosis. Expression of a kinase-inactive mutant or SLK small interfering RNAs inhibited cell proliferation and resulted in an accumulation of quiescent cells stimulated to re-enter the cell cycle in the G2 phase. Cultures expressing the mutant SLK displayed a normal pattern of cyclin D, E, and B expression during cell adhesion and spreading (Wagner, S., Flood, T. A., O’Reilly, P., Hume, K., and Sabourin, L. A. (2002) J. Biol. Chem. 277, 37685–37692). Because of its association with the microtubule network, we investigated whether SLK plays a role in cell cycle progression, a process that requires microtubule dynamics during mitosis. Consistent with microtubule association in exponentially growing cells, our results showed that SLK co-localizes with the mitotic spindle in cells undergoing mitosis. Expression of a kinase-inactive mutant or SLK small interfering RNAs inhibited cell proliferation and resulted in an accumulation of quiescent cells stimulated to re-enter the cell cycle in the G2 phase. Cultures expressing the mutant SLK displayed a normal pattern of cyclin D, E, and B expression but failed to down-regulate cyclin A levels, suggesting that they cannot proceed through M phase. In addition, these cultures displayed low levels of both phospho-H3 and active p34/cdc2 kinase. Overexpression of active SLK resulted in ectopic spindle assembly and the induction of cell cycle re-entry of Xenopus oocytes, suggesting that SLK is required for progression through G2 upstream of H1 kinase activation.

Cell cycle progression is monitored through kinase-mediated signal transduction and the binding of various cyclin proteins to their respective cyclin-dependent kinases (Cdks (2, 3)). The activity of a cyclin/Cdk complex is regulated by cycles of expression and destruction of the cyclin subunit (reviewed in Ref. 4). G1 progression is regulated, in part, by cyclins D and E and their respective cyclin-dependent kinases in a complex pathway that results in the reinitiation of cell cycle (reviewed in Ref. 5). Cyclin B synthesis initiates at the end of S phase (6, 7) and forms a complex with p34cdc2/cdk1. This complex has been termed MPF (maturation promoting factor or mitosis promoting factor) and is required for mitotic entry (reviewed in Ref. 8). During interphase, cytoplasmic MPF is kept inactive by inhibitory phosphorylation of cdc2 on Thr-14 and Tyr-15 by Myt1 and Wee1, respectively (9–11). Activation of this complex is triggered by the Cdc25C phosphatase through cdc2 dephosphorylation of Thr-14 and Tyr-15 (12–14). Following dephosphorylation of these residues, MPF is believed to phosphorylate and further activate Cdc25C, resulting in full activation of MPF through an autocatalytic feedback loop (15, 16). This results in the translocation of MPF from the cytoplasm to the nucleus at the beginning of mitosis (17), where it phosphorylates histone H1 (18) and induces changes in the microtubule network (19) and actin filaments (20).

In Xenopus, polo-like kinase (Plx1) has been shown to phosphorylate and activate Cdc25 (21), and polo-like kinase xPlkk1 has been shown to be a direct activator of Plx1 (22). However, this may be an organism-specific phenomenon since depletion of mammalian polo-like kinase (Plx1) results in elevated activity of Cdc2 (23), suggesting a role for Plk in mitotic progression rather than mitotic entry. To date, a bona fide upstream activator of mammalian Cdc25C has not been identified.

Chromosomal condensation is accompanied by the hyperphosphorylation of histone H1 (24) and phosphorylation of H3 on Ser-10 (25, 26). Microtubules then organize into a bipolar spindle and attach to the kinetochores of each sister chromosome. Chromosome attachment prior to segregation is monitored by the spindle checkpoint protein MAD2 (mitotic arrest-deficient), which binds kinetochores lacking microtubule attachment generating a “wait-anaphase” signal (reviewed in Ref. 27). Following the attachment of the last unattached kinetochore, the wait-anaphase signal is silenced, and the anaphase-promoting complex, in association with Cdc20, initiates chromosome segregation (reviewed in Ref. 28) and culminates into cytokinesis (reviewed in Ref. 29).

The murine Ste20-like kinase (SLK) is a 220-kDa serine/threonine kinase that was first demonstrated to induce actin remodeling and apoptosis in a wide range of cell lines (30, 31). The amino-terminal kinase domain of SLK is closely related to that of lymphocyte-oriented kinase (LOK) and xPlkk1. In addition, SLK bears a central microtubule and nuclear-associated protein domain and a carboxyl AT1–46 homology (termed ATH for AT1–46 homology) domain that is also found in LOK and xPlkk1 (30, 31). The function of both the nuclear-associated protein and the AT1–46 domains has yet to be elucidated. SLK is expressed early in development, preferentially in neuronal and myogenic lineages and ubiquitously in adult tissue (32). It has been shown to co-localize with the microtubule network.
with adhesion markers during cell spreading and is intimately linked to the microtubule network (1).

The observation that SLK is associated with the microtubule network (1) and that it was shown to phosphorylate and activate Plk (33) prompted us to investigate the role of SLK in cell cycle progression. Consistent with microtubule association in exponentially growing cells, our results showed that SLK co-localizes with the mitotic spindle in cells undergoing mitosis. Down-regulation of SLK protein levels by siRNA knockdown or expression of a kinase-inactive mutant (KAc) inhibited cell proliferation and resulted in a G2/M accumulation of quiescent cells induced to re-enter the cell cycle by serum stimulation. Cultures expressing the mutant SLK displayed a normal pattern of cyclin D, E, and B expression but failed to down-regulate cyclin A levels, suggesting that they cannot proceed through M phase. Supporting this, cells expressing the inactive SLK mutant failed to induce histone H3 phosphorylation and to fully activate p34/Cdc2. Finally, overexpression of an activated kinase resulted in ectopic spindle formation and activation of Plx1 in Xenopus extracts, suggesting that activated SLK induces cell cycle re-entry. Overall, our results suggested that SLK is required for progression through G1 upstream of Cdc2.

**EXPERIMENTAL PROCEDURES**

**Cell Lines, Culture, and Adenovirus Infection**—The mouse fibroblast lines MEF-3T3 (MEF Tet-Off, C3018, Clonetch) and C3H10T1/2 (ATCC number CCL-226) were used in all experiments. Similar results were obtained for both cell lines. The GFP-tubulin-expressing cells (LLCKP-1) were a kind gift from Patricia Wadsworth (34). Cell lines were maintained at 37 °C with 5% CO2 in Dulbecco's modified Eagle's medium (DMEM, BioWhittaker) supplemented with 10% fetal bovine serum (FBS), 2 mM l-glutamine, 50 μg/ml penicillin, and 50 μg/ml streptomycin. For cell cycle experiments, fibroblasts were arrested by 48-h incubations in 0.25% FBS-DMEM and released from quiescence by the addition of 20% FBS-DMEM. The epitope-tagged kinase-dead or activated versions of SLK used in these studies (HA-KAc or HA-YAc) have been described previously (1) and consist of a carboxyl-terminal truncation (amino acids 1–373) with or without an ATP-binding site (35). The SLK antibody was as described previously (1). Primary antibodies were detected using either a goat anti-rabbit IgG or a goat anti-mouse IgG horseradish peroxidase-labeled secondary antibody (Bio-Rad) and visualized using Western Lightning chemiluminescence reagent (PerkinElmer Life Sciences) and exposure to x-ray film. SLK immunoprecipitations and kinase assays were carried out as described previously (31). Briefly, equal amounts of lysate were immunoprecipitated for 2 h at 4 °C using 1 μg of SLK antibody and 20 μl of protein A-Sepharose 4 Fast Flow (Amersham Biosciences).

**Cell Counts, Cloning, and Transfections**—To monitor cell proliferation, the cells were counted after infection on day 0 at a multiplicity of infection of 100 as described above. Cell populations were trypsinized and scored by trypan blue exclusion over time. Cell counts were performed in triplicates in three independent experiments. For antisense SLK expression, a 5’ 300-bp SLK fragment was cloned into the pEMSV-
puro expression vector in the reverse orientation. The antisense SLK plasmid and the puromycin control were then transfected into MEF-3T3 cells using Lipofectamine™ 2000 (Invitrogen) according to the manufacturer’s protocol and selected in puromycin (100 μg/ml) over a 2-week period. Following selection, stable transfectants were visualized using CYTO-QUIK staining (Fisher Health Care) followed by several PBS washes. SLK Smart Pools siRNAs were obtained from Dharmacon against the following murine SLK target sequences: 5’-GGTTGAGATTGACATATTA-3’, in addition to a scrambled siRNA (5’-GATAATTATGGATGTGAC-3’). Control siRNA consisted of the siCONTROL (Dharmacon; 5’-UAGCGACUAAACACAUCAAAU-3’), having no perfect match to known human or mouse sequences. All siRNAs were transfected using the Transit-TKO reagent (Mirus Corp.) according to the manufacturer’s instructions.

RESULTS

SLK Co-localizes with the Mitotic Spindle and Is Regulated during the Cell Cycle—We have previously shown that a proportion of SLK is associated with the microtubule network of exponentially growing fibroblasts (1). During immunofluorescence studies involving the co-localization of SLK to the microtubule network in asynchronous cultures, we observed rare patterns of SLK and α-tubulin co-localization that resembled the mitotic spindle. To further investigate the possibility that SLK might co-localize with the mitotic spindle, we performed confocal microscopy. Although some SLK was found outside of the mitotic spindle, confocal analysis of DAPI-stained cells, in combination with anti-SLK and anti-α-tubulin, shows that most of the SLK staining co-localized with tubulin during metaphase (Fig. 1, A and B). The observation that SLK is associated with α-tubulin, a central component of the
were released from G0 by the addition of serum, collected at various stages of the cell cycle using synchronized cell populations. Serum-starved cultures infected with the KΔC adenovirus were pulse-chased and then released from G0 by the addition of 20% serum. Cells were then monitored by flow cytometric DNA content analysis. After 32 h of serum stimulation, HA-KΔC-expressing cells show a delay in G2/M transit time when compared with control LacZ-infected cells (Fig. 3A). Supporting this, BrdUrd labeling of exponentially growing H9004 cells expressing LacZ or HA-KΔC showed that they re-entered the G1 compartment, suggesting that HA-KΔC is able to interfere with SLK-dependent pathways.

**SLK is Required for Progression through G2**—To further investigate the potential role of SLK in proliferation, an expression vector bearing an antisense SLK fragment was transfected into MEF-3T3 fibroblasts and subjected to puromycin selection. Stable clones were visualized after 14 days using Cyto-Quick stain (Fisher). As shown in Fig. 2C, antisense SLK-transfected cultures reproducibly displayed a marked reduction in colony numbers when compared with the pEMSV-puro control vector. Furthermore, expansion and Western blot analysis of the resulting antisense SLK clones showed that they did not down-regulate SLK, suggesting that the antisense RNA was not expressed in those clones (not shown).

As for antisense SLK expression, infection of MEF-3T3 cells with an adenoviral vector carrying a kinase-dead truncation of SLK (SLK1–373K63R, termed HA-KΔC) suppressed cell proliferation as determined by dye exclusion cell counts (Fig. 3D). Taken together, these results indicate that SLK activity is required for cell proliferation and that our truncated kinase-dead version, HA-KΔC, is able to interfere with SLK-dependent pathways.

**Kinase-deficient SLK Inhibits Proliferation**—To investigate the potential role of SLK in proliferation, an expression vector bearing an antisense SLK fragment was transfected into MEF-3T3 fibroblasts and subjected to puromycin selection. Stable clones were visualized after 14 days using Cyto-Quick stain (Fisher). As shown in Fig. 2C, antisense SLK-transfected cultures reproducibly displayed a marked reduction in colony numbers when compared with the pEMSV-puro control vector. Furthermore, expansion and Western blot analysis of the resulting antisense SLK clones showed that they did not down-regulate SLK, suggesting that the antisense RNA was not expressed in those clones (not shown).

As for antisense SLK expression, infection of MEF-3T3 cells with an adenoviral vector carrying a kinase-dead truncation of SLK (SLK1–373K63R, termed HA-KΔC) suppressed cell proliferation as determined by dye exclusion cell counts (Fig. 3D). Taken together, these results indicate that SLK activity is required for cell proliferation and that our truncated kinase-dead version, HA-KΔC, is able to interfere with SLK-dependent pathways.

**SLK Is Required for Progression through G2**—To further investigate the potential role of SLK in cell cycle progression, its activity was monitored throughout the different phases of the cell cycle using synchronized cell populations. Serum-starved cultures were released from G0 by the addition of serum, collected at various times, and monitored for SLK activity and DNA content by kinase assays and fluorescence-activated cell sorter analysis, respectively. Fig. 2A displays the cell cycle phase as determined by flow cytometric measurements of DNA content in these synchronized populations following serum stimulation. After 24 h of serum stimulation, a marked and consistent 3–4-fold increase in SLK kinase activity (Fig. 2B) was observed when ~60–70% of the cells entered the G2/M compartment, as determined by fluorescence-activated cell sorter analysis. Similarly, an increase in SLK activity during G2 has been reported previously, albeit to a much lesser extent (~1.3-fold) (33). Interestingly, as the cells exited G2, a marked reduction in kinase activity was observed. The total levels of SLK protein were found to be unaffected throughout the time course. These results suggested that SLK kinase activity is up-regulated at a time point at which the vast majority of the cells display a 4N DNA content.

**FIGURE 3. Expression of kinase-inactive SLK results in a G2/M block.** C3H10T1/2 cells infected with adenovirus carrying LacZ or HA-KΔC were synchronized to quiescence by serum deprivation and then released by the addition of 20% serum. Cells were then monitored by flow cytometric DNA content analysis. After 32 h of serum stimulation, HA-KΔC-expressing cells show a delay in G2/M transit time when compared with control infected cultures (A and B). Supporting this, BrdUrd labeling of exponentially growing cultures and DNA content monitoring of BrdUrd-positive cells show that HA-KΔC-infected cells proceed through G2/M with delayed kinetics (C). A representative of four independent experiments is shown.
SLK Is Required for Cell Cycle Progression

Our results showed that expression of a kinase-inactive SLK is sufficient to induce an early G₂ cell cycle block in cycling fibroblasts, suggesting that an SLK-dependent pathway is required during G₂ for progression into mitosis. To determine whether SLK plays a central role in G₂ and to rule out potential nonspecific effects by KΔC overexpression, exponentially growing fibroblasts were transfected with an SLK siRNA pool and subjected to DNA content analysis. Transfection of SLK siRNAs down-regulated SLK protein levels by 80–90% within 48 h (Fig. 6A). No effect was observed in the siRNA control samples. DNA content analysis 48 h following siRNA transfection showed that SLK knockdown resulted in a marked G₂/M accumulation (92% 4N DNA content; Fig. 6B), an inhibition of proliferation, and increased cyclin A levels (data not shown). These data further support a role for SLK in cell cycle progression and rule out potential nonspecific effects by KΔC overexpression. Supporting these results, microinjection of activated SLK (SLK1–373) in GFP-tubulin labeled cells induced ectopic mitotic spindles in the injected cells within 3–6 h, ultimately resulting in cell death (Fig. 6C). Similarly, injection of the activated SLK, but not kinase-dead, mRNA in Xenopus oocytes resulted in the hyperphosphorylation of Plx1 and GVBD without progesterone induction, suggesting that the injected eggs re-entered the cell cycle as for progesterone-treated oocytes (Fig. 6D). Interestingly, expression of a kinase-dead version followed by progesterone treatment did not inhibit cell cycle re-entry and GVBD (not shown), suggesting that alternative pathways, such as the mitogen-activated protein kinase (MAPK) pathway, may be sufficient to promote maturation (39).

DISCUSSION

Cell cycle progression is regulated by complex signaling networks involving post-translational modification, gene expression, and cytoskeletal reorganization. Progress through the various phases involves the activation of key factors and is monitored by various checkpoint proteins. We have previously isolated an Ste20-like kinase termed SLK that is involved in cytoskeletal reorganization (1, 30, 31). Interestingly, a fraction of SLK protein is also observed to associate with the microtubule network in spreading and exponentially growing cells (1). Here we have shown that SLK also associates with the microtubule network at mitosis, suggesting that it plays a role in cell cycle progression. To investigate this, the activity and expression of SLK was evaluated throughout the cell cycle, and the effect of a kinase-defective SLK on cell cycle progression was assessed.

Our results showed that SLK co-localizes with the mitotic spindle during M phase and that its kinase activity is up-regulated as synchronized fibroblast cultures enter the G₂/M compartment. Expression of an following stimulation, when a significant proportion of the cells entered S phase (Fig. 2A), and down-regulated thereafter, suggesting that both cultures entered and exited S phase with similar kinetics. In addition, both cultures induced cyclin B expression at ~8–16 h. Similarly, both cultures induced cyclin A at the G₁/S transition. However, only control-infected cultures were found to differ markedly between LacZ- or KΔC-infected cultures. However, the levels of cyclin A protein in KΔC-expressing cells were found to remain elevated (arrowheads), suggesting a G₂ phase block. Infection was confirmed by the HA tag of KΔC, and even loading was evaluated by actin levels. Western blot analysis of synchronized fibroblasts transiting through G₀/M following infection with HA-KΔC virus or LacZ control and serum stimulation. A marked reduction in CDK2 activation was observed in KΔC-expressing cultures. The righthand lane represents a control M phase-synchronized extract.
FIGURE 5. KΔC expression inhibits histone H3 phosphorylation. Serum-starved C3H10T1/2 cells were infected with LacZ (A, B, and C) or KΔC (D, E, and F) encoding viruses and then serum-stimulated for 24, 28, or 32 h. Cells were fixed and stained for anti-HA (D) or β-galactosidase (β-Gal) (A) in conjunction with anti-phospho-H3 (B and E). Nuclei were visualized by DAPI counterstaining (C and F). A marked reduction in pH3 staining was observed in KΔC-expressing cells. G, serum-starved C3H10T1/2 cells were stimulated with 20% fetal calf serum and double-stained for phospho-H3 and HA, or LacZ, at the indicated time points (in hours). Double positive HA (or LacZ) and phospho-H3 cells were scored. The results are shown for three independent infections for which at least 200 nuclei were counted.
SLK is Required for Cell Cycle Progression

FIGURE 6. Role for SLK in G2 progression. Exponentially growing C3H10T1/2 were transfected with the SLK siRNA pool or siCONTROL and analyzed by Western blot for SLK expression (A) and by flow cytometry for DNA content (B) 48 h after transfection. The samples labeled control correspond to cells transfected with the Dharmacon siCONTROL RNA. Identical results were obtained with a SLK scrambled siRNA (not shown). A marked down-regulation of SLK at 50 nM of siRNA resulted in the accumulation of the cells in the G2/M compartment, further supporting a requirement for SLK for progression through G2. C, exponentially growing LLCPK-1 fibroblasts expressing GFP-tubulin were microinjected with an activated form of SLK (HA-tagged; amino acids 1–373). All (n = 25) injected cells (arrowheads), detected by anti-HA staining, displayed ectopic mitotic spindles when expressing activated SLK (panels i and iii; merged fluorescence), suggesting that SLK induces mitotic entry. No spindle formation was observed when cells were injected with the kinase-dead version (panels ii and iv). D, Xenopus oocytes injected with the same form of activated SLK re-entered the cell cycle, as evidenced by GVBD and the shift in the molecular weight of Plx1, indicative of phosphorylation (pPlx1), suggesting that SLK can activate mitotic entry in Xenopus eggs. As a control, activation of Plx1 by progesterone (lane P) was used (lane C = untreated). Expression of kinase-dead SLK (mSLK-KD) could not induce oocyte maturation. The GVBD data represent the average of four independent experiments. Western blot analysis shows the expression of the Myc-tagged SLK protein.

An interpretation of the data presented here indicated that SLK is required during G2, at a point after cyclin A expression and before Cdc2 activation, for progression into mitosis. A Xenopus LOK/Stx10 homolog, xPolo-like kinase kinase (xPlkk1), has been demonstrated to activate Plx1 (22). Interestingly, SLK has been demonstrated to phosphorylate and activate Plx1 in a mammalian system (33). However, we and others4 have not observed phosphorylation and activation of mammalian Plk1 by SLK in in vitro kinase assays or transient transfections (not shown).

An evolutionary conserved relationship appears to exist between mammalian and amphibian Ste20-like kinases, the polo-like kinase family, and the Cdc25C phosphatase. The human homolog of the Ste20-like kinase LOK, Stx10, has been shown to associate with and phosphorylate Plk1 (40), and Plk3 has been shown to initiate the nuclear translocation of Cdc25C by phosphorylating Cdc25C on serine 191 (41). In Xenopus, the polo-like kinase, Plx1, has been shown to activate Cdc25C (21), an event that has not been unarguably proven in a mammalian system and may be species-specific. If Plk requires a signal from SLK to activate Cdc25C in a mammalian system, one would predict a G2/M arrest, similar to the findings presented here. This would result in a Cdc25C protein that is deficient in all of the post-translational modifications required to initiate mitosis. However, the questionable validity and specificity of phospho-specific Cdc25C antibodies has made the identification of the various forms of the phospho-Cdc25C difficult and inconclusive (not shown). We have previously shown that SLK can induce actin depolymerization and cell death in various cell lines (1, 31). One possibility is that SLK overexpression in cycling cells induces a deregulated mitotic entry, bypassing cell cycle controls, resulting in actin breakdown and death. Alternatively, SLK-mediated cytoskeletal reorganization may be required for, or trigger, checkpoint activation, G2 progression, and mitotic entry.

Overall, we have shown that SLK is a component of the mitotic spindle and that its activity is required in G2, upstream of Cdc2, for efficient progression into mitosis. The characterization of the molecular components of SLK-dependent signaling pathways now awaits the identification of its substrates and binding proteins.

Acknowledgment—We are grateful to Patricia Wadsworth for providing the LLCPK-1 cells expressing GFP-tubulin.

*P. Stambrook, personal communication.
REFERENCES

1. Wagner, S., Flood, T. A., O'Reilly, P., Hume, K., and Sabourin, L. A. (2002) J. Biol. Chem. 277, 37685–37692
2. Evans, T., Rosenthal, E. T., Youngblom, J., Distel, D., and Hunt, T. (1983) Cell 33, 389–396
3. Pines, J. (1995) Biochem. J. 308, 697–711
4. Smits, V. A., and Medema, R. H. (2001) Biochim. Biophys. Acta. 1519, 181–189
5. Piaggio, G., Farina, A., Perrotti, D., Manni, I., Fuschi, P., Sacchi, A., and Gaetano, C. (1995) Exp. Cell Res. 216, 396–402
6. Pines, J., and Hunter, T. (1989) Cell 58, 833–846
7. Nurse, P. (1990) Nature 344, 503–508
8. Liu, F., Stanton, J. J., Wu, Z., and Piwnica-Worms, H. (1997) Mol. Cell. Biol. 17, 571–583
9. Lee, M. S., Ogg, S., Xu, M., Parker, L. L., Donoghue, D. J., Maller, J. L., and Piwnica-Worms, H. (1992) Mol. Biol. Cell 3, 73–84
10. Hoffmann, I., Clarke, P. R., Marecotte, M. J., Karsenti, E., and Draetta, G. (1993) EMBO J. 12, 53–63
11. Lee, M. S., Ogg, S., Xu, M., Parker, L. L., Donoghue, D. J., Maller, J. L., and Piwnica-Worms, H. (1992) Mol. Cell. Biol. 3, 73–84
12. Roth, S. Y., Collini, M. P., Draetta, G., Beach, D., and Allis, C. D. (1991) EMBO J. 10, 2069–2075
13. Blangy, A., Lane, H. A., d’Herin, P., Harper, M., Kress, M., and Nigg, E. A. (1995) Cell 83, 1159–1169
14. Yamashiro, S., Yamakita, Y., Ishikawa, R., and Matsumura, F. (1990) Nature 344, 675–678
15. Kumagai, A., and Dunphy, W. G. (1996) Science 273, 1377–1380
16. Qian, Y. W., Erikson, E., and Maller, J. L. (1998) Science 282, 1701–1704
17. Liu, X., and Erikson, R. L. (2002) Proc. Natl. Acad. Sci. U. S. A. 99, 8672–8676
18. Bradbury, E. M., Inglis, R. J., Matthews, H. R., and Sarner, N. (1973) Eur. J. Biochem. 33, 131–139
19. Allis, C. D., and Gorovsky, M. A. (1981) Biochemistry 20, 3828–3833
20. Hendzel, M. J., Wei, Y., Mancini, M. A., Van Hooser, A., Ranalli, T., Brinkley, B. R., Bazett-Jones, D. P., and Allis, C. D. (1997) Chromosoma (Berl.) 106, 348–360
21. Shah, J. V., and Cleveland, D. W. (2000) Cell 103, 997–1000
22. Peters, J. M. (2002) Mol. Cell 9, 931–943
23. Scholey, J. M., Brust-Mascher, I., and Mogilner, A. (2003) Nature 422, 746–752
24. Sabourin, L. A., and Rudnicki, M. A. (1999) Oncogene 18, 7566–7575
25. Sabourin, L. A., Tamai, K., Seale, P., Wagner, J., and Rudnicki, M. A. (2000) Mol. Cell. Biol. 20, 684–696
26. Zhang, Y. H., Hume, K., Cadonic, R., Thompson, C., Hakim, A., Staines, W., and Sabourin, L. A. (2002) Brain Res. Dev. Brain Res. 139, 205–215
27. Ellinger-Ziegelbauer, H., Karasuyama, H., Yamada, E., Tsujikawa, K., Todokoro, K., and Nishida, E. (2000) Genes Cells 5, 491–498
28. Runan, N. M., Fagerstrom, C. J., Yvon, A.-M. C., and Wadsworth, P. (2001) Mol. Biol. Cell 12, 971–980
29. Vicogne, J., Cailliau, K., Tulasne, D., Browaeys, E., Yan, Y. T., Fafur, V., Vilain, J. P., Legrand, D., Tiolet, J., and Dizousis, C. (2004) J. Biol. Chem. 279, 37407–37414
30. Geley, S., Kramer, E., Gieffers, C., Gannon, J., Peters, J. M., and Hunt, T. (2001) J. Cell Biol. 153, 137–148
31. den Elzen, N., and Pines, J. (2001) J. Cell Biol. 153, 121–136
32. Pines, J., and Rieder, C. L. (2001) Nat. Cell Biol. 3, E3–6
33. Schmitt, A., and Nebreda, A. R. (2002) J. Cell Sci. 115, 2457–2459
34. Walter, S. A., Cutler, R. E., Jr., Martinez, R., Gishizky, M., and Hill, R. J. (2003) J. Biol. Chem. 278, 18221–18228
35. Bahassi, M., Hennigan, R. F., Myer, D. L., and Stambrook, P. J. (2004) Oncogene 23, 2658–2663