A Mammalian Homolog of Fission Yeast Cdc5 Regulates G2 Progression and Mitotic Entry*

(Received for publication, October 1, 1997, and in revised form, December 2, 1997)

Harold S. Bernstein‡∥§ and Shaun R. Coughlin‡

From the ‡Cardiovascular Research Institute and Departments of §Pediatrics and ¶Medicine and Cellular and Molecular Pharmacology, University of California, San Francisco, California 94143

Progression through G2/M of the mammalian cell division cycle requires the coordinated expression of many gene products, but little is known of the transcriptional regulators involved. Schizosaccharomyces pombe Cdc5 is a putative transcription factor implicated in G2/M transit. We recently identified a cDNA encoding a putative human transcription factor, now designated human Cdc5 (hCdc5), with homology to S. pombe Cdc5. Widespread expression of hCdc5 in human tissues and homology with expressed sequences in other eukaryotes suggested an evolutionarily conserved general function. Nuclear import of hCdc5 upon serum stimulation of mammalian cells suggested a possible role in cell proliferation. We now report that overexpression of hCdc5 in mammalian cells shortened G2 and reduced cell size. A dominant negative mutant of hCdc5 lacking the carboxyl-terminal activation domain slowed G2 progression and delayed entry into mitosis. Thus, hCdc5 is the first transcriptional regulator shown to affect G2 progression and mitotic entry in mammalian cells.

Orchestration of the cell division cycle includes a series of checkpoints which ensure that some events are completed before others begin (1). One set of controls determines whether the cell replicates its genome in preparation for division (G1/S), whereas another checks that DNA replication is complete and that the cell has grown sufficiently for division to take place (G2/M) (2). Cyclins and cyclin-dependent kinases mediate these events and are themselves regulated through several mechanisms (3). In fission yeast, regulation of the Cdc2-Cdc13 cyclin complex by the Wee1 kinase and Cdc25 phosphatase is thought to be the primary mechanism controlling G2/M (4, 5). The Cdc2-Cdc13 complex accumulates during S phase, but Cdc2 is phosphorylated and thereby maintained in an inactive state by Wee1 (6, 7). As cells complete DNA replication, Cdc25 dephosphorylates Cdc2 (8, 9), heralding progression through G2 and entry into mitosis. Upstream regulators of Wee1 and Cdc25 also have been described. Nim1 kinase inactivates Wee1 through phosphorylation (10–12), whereas protein phosphatase 2A has been shown to negatively regulate mitotic entry by its interaction with Wee1 and/or Cdc25 (13).

The biochemical events controlling G2/M transit in mammalian cells are remarkably similar to those in S. pombe. Mammalian Cdc2 kinase accumulates in S phase (14) and is regulated by a Wee1 kinase (15) and Cdc25 phosphatase (16, 17). Less is known, however, about their upstream regulation. More generally, whereas G2/M progression clearly requires the coordinated expression of many gene products, little is known about how these are regulated at the level of transcription. Characterization of transcription factors regulating G2 progression and mitotic entry would significantly advance our understanding of the mechanisms controlling this portion of the cell cycle.

The cdc5 gene product in fission yeast is a putative transcription factor implicated in G2/M transit (18, 19). Haploid yeast bearing a temperature-sensitive mutation in the cdc5 gene arrest with a diploid complement of DNA, single nucleus, and decondensed chromosomes without evidence of mitotic arrest or defective DNA replication (18, 19). We recently identified a cDNA encoding a putative human transcription factor (20) with homology to S. pombe Cdc5 (19). The amino acid sequence of this new protein was 75% identical to that of Cdc5 over 223-amino-terminal residues containing the DNA binding domain, but this identity decreased to 17% over the subsequent 535 amino acids (20). Its widespread expression in human tissues and homology with expressed sequences in other eukaryotes suggested a possible role in cell proliferation (20). Without clear evidence that this was a functional Cdc5 homolog, however, the new protein initially was designated Pombe Cdc5-related protein.

We now describe the functional analysis of Pombe Cdc5-related protein in mammalian cells. These studies strongly suggest that it is a human homolog of S. pombe Cdc5, and so we henceforth refer to it as hCdc5. Overexpression of hCdc5 shortened G2 and reduced cell size. A dominant negative mutant of hCdc5 lacking the carboxyl-terminal activation domain slowed G2 progression and delayed entry into mitosis. These results demonstrate that hCdc5 regulates G2 progression and mitotic entry and is the first transcriptional regulator shown to effect G2/M transit in mammalian cells.

EXPERIMENTAL PROCEDURES

Cell Transfections and Generation of Stable Lines—All cell lines were obtained from ATCC and maintained according to their recommendations. COS-7 and BALB/c3T3 cells were transfected using LipofectAMINE (Life Technologies, Inc.) according to the manufacturer’s instructions. Jurkat cells were transfected by electroporation at 0.25 V, 960 microfarads. Synchronization of BALB/c3T3-derived cultures in G0 was accomplished by washing cells with serum-free media and maintaining in media containing 0.5% bovine calf serum for 48 h. Release into G1 was accomplished by the addition of prewarmed bovine calf

* This work was supported in part by National Institutes of Health Grants HL44907 and HL43821. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

∥ Supported by National Institutes of Health Clinical Investigator Development Award HL03228. To whom correspondence should be addressed: University of California, San Francisco, 513 Parnassus Ave., Box 0632, San Francisco, CA 94143-0632. E-mail: HS_Bernstein@pedcardgateway.ucsf.edu

§ The abbreviations used are: hCdc5, human Cdc5; GFP, green fluorescent protein; CMV, cytomegalovirus.
serum to 10%. The plasmid encoding FLAG-tagged hCdc5 in pcDNA3 (Invitrogen) was constructed as described previously (20). Cell line 3T15.8 was generated by transfecting BALBc/3T3 cells with pUHD 15–1 (Display Systems Biotechnology), which directs the expression of a tetracycline repressor-VP16 fusion protein, then selecting in the presence of 400 μg/ml G418. Cell line 3T15.8.22 was made by co-transfecting 3T15.8 cells with plasmids encoding FLAG-tagged hCdc5 downstream from a minimal CMV promoter and seven tandem repeats of the tet operator (pUHD 10–1; Display Systems Biotechnology) and a marker for hygromycin resistance. Transfectants were selected with

**FIG. 1.** Constitutive overexpression of hCdc5 accelerates G2/M progression. Panel A, inducible expression of hCdc5. Whole cell lysates were analyzed by immunoblotting with monoclonal antibodies to endogenous hCdc5 (B1) and recombinant, FLAG-tagged hCdc5 (M2). Recombinant hCdc5, absent from the 3T15.8 parental line, was expressed at levels ~2-fold above endogenous protein by 36 h after tetracycline (Tet) withdrawal (3T15.8 versus 3T15.8.22 · Tet × 36 h). A nonspecific band (*) recognized by goat anti-mouse antibody served as a control for protein loaded. Panel B, cell cycle progression with hCdc5 overexpression. Synchronized 3T15.8.22 cultures were analyzed for DNA content at timepoints indicated. A representative study is shown (n = 5). A more rapid return to G1 is apparent in the absence of tetracycline. Panel C, growth acceleration with hCdc5 overexpression. Cell number was counted in actively dividing cultures at indicated timepoints. Data shown are mean ± S.E. (n = 4). Accelerated growth over several cell cycles was observed for line 3T15.8.22 in the absence of tetracycline (p < 0.05). Panel D, phase distribution with hCdc5 overexpression. Asynchronously dividing cultures ± tetracycline were analyzed for cell cycle distribution. Phase lengths were calculated from the percentage of cells in each phase and the calculated cycle lengths (Fig. 1C). Data shown are mean ± S.E. (n = 4). The length of G2/M in 3T15.8.22 cells was significantly decreased in the absence of tetracycline (p < 0.01).

**FIG. 2.** Overexpression of hCdc5 causes a decrease in cell size. Dividing cultures were synchronized in low serum for 48 h, then stimulated with serum. Forward light scatter (FSc) as a function of cell size was measured at timepoints indicated. Data shown are mean ± S.E. (n = 4). Average cell size of 3T15.8.22 cultures decreased as they completed a cycle in the absence of tetracycline. Upon stimulation with serum, 3T15.8.22 cells remained smaller in the absence of tetracycline, with no significant increase in size over the course of the cell cycle compared with cells grown in the presence of tetracycline (p < 0.01).
200 μg/ml hygromycin in the presence of G418 and 10 μg/ml tetracycline. To assess the effects of constitutive hCdc5 overexpression, cultures were rinsed with tetracycline-free media and re-fed with the same media every 2 h for 6 h. Assays were performed after 36–48 h in tetracycline-free media. pNGFP-N1, encoding GFP with P64L and S65T mutations (21), was obtained from CLONTECH.

Antibodies and Immunoblot Analysis—Monoclonal antibody B1 was generated against a peptide representing the carboxyl terminus of hCdc5 (residues 769–788) (20) according to standard methods (22). Monoclonal antibody M2 directed against the FLAG epitope was obtained from Eastman Kodak Co. Polyclonal antibodies recognizing murine cyclins were obtained from Santa Cruz Biotechnology. Immunoblot analysis was performed as described previously (20). Monoclonal antibody B1, which recognized both recombinant and endogenous hCdc5, was used to compare expression levels of both proteins. Densitometric analysis was accomplished by scanning representative ECL results into Photoshop (Adobe), then measuring signal densities using NIH Image (NIH).

Transcription Reporter Assay—Portions of the hCdc5 coding region were cloned in-frame into pM (CLONTECH), downstream of nucleotides 1–441 of GAL4. These fusion constructs were co-transfected with a reporter plasmid, pFR-Luc (Stratagene), containing the entire coding region of Photinus pyralis (firefly) luciferase downstream of five tandem repeats of the GAL4 binding element and a basic promoter (TATATA). COS-7 cells were transfected as described above, and luciferase activity was assayed in whole cell lysates using the dual-luciferase reporter assay system (Promega) according to the manufacturer’s instructions in a Monolight 3010 luminometer (Analytical Luminescence Laboratory). Plasmid pRL-SV40 (Promega), encoding Renilla reniformis (sea pansy) luciferase downstream of an SV40 promoter, was included in each transfection for normalization of transfection efficiency. Plasmid pM3VP16 (CLONTECH), encoding a GALA-VP16 fusion, was used as a positive control.

Cell Cycle Analysis—Asynchronously dividing or synchronized cultures were analyzed after 72 h of active division or at indicated time-points after serum stimulation, respectively. 106 cells were stained with propidium iodide and Hoechst 33342 to allow measurement of DNA content in living cells. Flow cytometry was performed using a Becton Dickinson FACStar Plus with dual argon ion lasers at 488 and 363 nm light output. Propidium iodide, Hoechst, and GFP signals were acquired using 630/22-, 470/10-, and 530/30-nm bandpass filters, respectively. All analyses of DNA content were performed on 20,000 collected events using CellQuest software (Becton Dickinson). Propphase index assay was accomplished by the addition of 0.5 μg/ml nocodazole 48 h after transfection. Cells then were stained with Hoechst 33342 at various timepoints. Slides containing 5 × 105 cells were prepared in triplicate from each transfection at each timepoint, and 200 GFP-positive cells per slide were scored for the presence of chromosomal condensation. For all experiments, analysis of variance was used to identify significant differences among all groups. Multiple comparisons were then performed using the Bonferroni t test to isolate specific differences.

RESULTS AND DISCUSSION

To study the effects of hCdc5 on the mammalian cell division cycle, we attempted its constitutive overexpression in a variety of cell types but were unable to generate stable lines. As this suggested that constitutive expression at high levels might be incompatible with cell survival, we generated a BALBc/3T3-based cell line, designated 3T15.8.22, in which hCdc5 was expressed under control of a tetracycline-regulatable promoter (23, 24). Recombinant hCdc5 was not detectable in the presence of tetracycline; however, in the absence of tetracycline, recombinant hCdc5 was expressed at levels ~2-fold above that of the endogenous protein (Fig. 1A), as determined by densitometry.

![Fig. 3: hCdc5 is expressed early in the cell cycle. Panel A, hCdc5 expression in synchronized cells. BALBc/3T3 cultures were made quiescent in low serum for 48 h, then stimulated with serum and harvested at indicated timepoints. Whole cell lysates were analyzed by immunoblotting with anti-hCdc5 monoclonal antibody (B1) or polyclonal antibodies to murine cyclins E (p50 CLNE), A (p60 CLNA), or B1 (p62 CLNB1). Although hCdc5 is barely detectable in quiescent cells, it is almost uniformly expressed in other phases of the cell cycle. Similar analysis of recombinant hCdc5 in 3T15.8.22 cultures in the absence of tetracycline demonstrated that the induced expression of recombinant hCdc5 paralleled that of the endogenous protein (data not shown). Panel B, cell cycle synchronization. Cells at each timepoint were fixed in ethanol, stained with propidium iodide, and evaluated by flow cytometry for DNA content distribution to confirm synchronization. 2N and 4N refer to DNA content.](Image 78x342 to 278x619)

![Fig. 4: Mutant hCdc5 fails to activate transcription in vitro. Panel A, schematic representation of hCdc5 and Δ675 deletion mutant constructs. NLS, nuclear localization signal. Panel B, transcription reporter assay. COS-7 cultures were co-transfected with hCdc5 fusion constructs, pFR-Luc reporter plasmid, and pRL-SV40 plasmid to normalize for transfection efficiency. Plasmids containing the GAL4 DNA binding domain alone and a GALA-VP16 activation domain fusion were used as negative and positive controls, respectively. Number ranges designate nucleotide sequence (20). Data shown are mean ± S.E. (n = 3). The amino-terminal portion of hCdc5 (GAL4 hCdc5(432–675)), present in mutant Δ675, failed to activate transcription in the absence of the carboxyl terminus.](Image 242x31 to 559x197)
As has been shown for transiently expressed (20) and endogenous hCdc5,2 stably expressed recombinant HcDc5 translocated to the nucleus upon serum stimulation (data not shown).

To assess the effect of increased hCdc5 expression on cell cycle progression, 3T15.8.22 cells were synchronized in G0 with low serum then stimulated with high serum and measured for DNA content at various times (Fig. 1B). Induction of hCdc5 by removal of tetracycline accelerated progression through the cell cycle; cells returned to G1 by 18 h after release from quiescence in the absence of tetracycline compared with 24 h in the presence of tetracycline. By contrast, the parent cell line 3T15.8, which expressed the tetracycline repressor-VP16 fusion protein but not exogenous hCdc5, returned to G1 in 24 h in the presence or absence of tetracycline (data not shown). This cell cycle acceleration was maintained over time, as manifest by an increase in cell number over several cycles (Fig. 1C). Over 72 h of continuous growth, cells overexpressing hCdc5 exhibited an abbreviated cell cycle length of ~19 h, whereas cells in which recombinant HcDc5 expression was repressed by tetracycline grew at a slower rate, with a cycle length of ~28 h. The cell cycle length of 3T15.8 cultures was ~26 h and unaffected by tetracycline.

To determine which phase of the cell cycle was shortened by overexpression of hCdc5, we analyzed asynchronously dividing 3T15.8.22 and 3T15.8 cultures grown in the presence and absence of tetracycline (Fig. 1D). In 3T15.8.22 cultures, a highly significant tetracycline-dependent decrease in the calculated length of G2/M was noted (1.9 h versus 7.4 h). An effect in S phase also was seen (2.2 h versus 5 h). Control 3T15.8 cultures showed no significant tetracycline-dependent effect. Thus, hCdc5 overexpression significantly accelerated G2/M.

In fission yeast, cells must reach a size threshold to enter mitosis (5, 25). In mammalian cells, size requirements for mitotic entry are presumed but have not been demonstrated. Since mammalian cells overexpressing hCdc5 displayed a remarkable acceleration of G2/M, we asked whether overexpression might affect cell size. Dividing 3T15.8.22 cultures were synchronized in G0 with low serum in the presence or absence of tetracycline, then stimulated with serum and analyzed over time for DNA content and cell size by flow cytometry (Fig. 2). In the absence of tetracycline, 3T15.8.22 cultures showed a decrease in average cell size as they completed a division cycle and entered G0. Upon stimulation with serum, this smaller average cell size was maintained over at least two cell cycles. Moreover, unlike the parent cell line or 3T15.8.22 cultures in the presence of tetracycline, cells overexpressing hCdc5 exhibited little increase in size as they approached mitosis.

The basis for the decrease in cell size with overexpression of hCdc5 is unknown. In fission yeast, wee1 mutants and Cdc25 overexpressors display shortened G2 and decreased cell size but a normal cell cycle length (5), supporting the notion that a size set point has been altered. In mammalian cells overexpressing hCdc5, decreased cell size occurred in the setting of a shortened cell cycle. A similar phenomenon has been observed

\[^{2}\] H. S. Bernstein, unpublished observation.

---

FIG. 5. Mutant hCdc5 specifically delays mitotic entry. Panel A, phase distribution in transfected Jurkat cells. Jurkat cultures were transfected with pNGFP-N1 and hCdc5, Δ675, or pcDNA3 and GFP-positive cells were analyzed. A representative experiment is shown (n = 3). Transient expression of hCdc5 and Δ675 resulted in a distribution toward G2/M, whereas expression of hCdc5 resulted in a distribution toward G1 relative to cells transfected with pcDNA3. Panel B, antagonistic effect of hCdc5 and Δ675. Cells were transfected with indicated amounts of plasmid (µg/10⁶ cells) and analyzed as in A. Data shown are mean ± S.E. (n = 3). An increase or decrease in the fraction of cells in G2/M was

seen with Δ675 (p < 0.05) or hCdc5 (p < 0.05), respectively, compared with pcDNA3 or untransfected cells. hCdc5 antagonized the effect of Δ675, suggesting that Δ675 acts as a dominant negative. Panel C, prophase index assay. Cells were transfected as in A, treated with nocodazole, and scored for chromosomal condensation. Prophase index is plotted as a function of time. Data shown are mean ± S.E. (n = 2). In the presence of nocodazole, cells accumulated in prophase at a slower rate when transfected with Δ675 (p < 0.01) and at a faster rate with hCdc5 (p < 0.05) compared with pcDNA3.
A Role for hCdc5 in G2 Progression and Mitotic Entry

in mammalian cells overexpressing cyclin D1. In these cells, decreased cell size was noted in the context of an uncompensated decrease in G1/S transit time and growth acceleration (26). The size reduction seen in hCdc5 overexpressing cultures may thus be due either to a readjustment of the size requirements for mitotic entry or, more simply, to a shortened cell cycle length with less time for cell growth.

Constitutive expression of recombinant hCdc5 at levels approximately 2-fold that of the endogenous protein in dividing cultures resulted in a surprisingly dramatic phenotype. If endogenous hCdc5 levels varied throughout the cell cycle, constitutive overexpression might result in relative changes in hCdc5 levels much greater than 2-fold during certain phases of the cell cycle. To investigate this possibility, we performed immunoblot analysis of synchronized BALB/c3T3 and 3T15.8.22 cultures with antibodies against endogenous and recombinant hCdc5. This demonstrated that endogenous hCdc5 was expressed early in the cell cycle (Fig. 3A), suggesting that its effects in G2/M are not regulated at the level of expression. As little else is known about the regulation of this recently described protein and its effectors, the mechanism by which hCdc5 overexpression influences cell growth parameters remains to be elucidated.

To further examine the role of hCdc5 in G2 progression, we generated a mutant, designated Δ675, that contained the DNA binding and nuclear localization domains of hCdc5 but lacked its carboxyl terminus (Fig. 4A). Studies of the carboxyl terminus of hCdc5 fused to a heterologous DNA binding domain suggested that this region contained a domain capable of activating transcription in vitro (Fig. 4B). Therefore, mutant Δ675 should compete with wild-type hCdc5 for binding to as yet unidentified target promoters but should fail to activate them. Jurkat human T cell leukemia cultures were transiently transfected with a vector directing expression of wild-type hCdc5 or Δ675 together with a plasmid directing expression of GFP as a marker for transfection (21). Cells transfected with the Δ675 cDNA expressed a protein of the expected molecular weight by immunoblot, and immunostaining confirmed that the mutant protein localized to the nucleus (data not shown). GFP-positive cells in asynchronously dividing cultures were analyzed for cell cycle distribution by flow cytometry (Fig. 5A). Cells expressing the Δ675 mutant accumulated in G2/M, whereas cells transfected with the empty vector had a phase distribution similar to untransfected cells. Cultures transfected with wild-type hCdc5 distributed toward G1, consistent with the experiments with stable lines described above. Co-expression of wild-type hCdc5 with Δ675 titrated the effect of the mutant on G2/M accumulation (Fig. 5B), consistent with the mutant acting specifically in the hCdc5 pathway.

We next used mutant Δ675 to ask whether inhibition of hCdc5 function delayed G2 versus mitosis itself (Fig. 5C). Jurkat cultures transfected as above were examined for chromosomal condensation at various times after exposure to nocodazole, which arrests these cells in prophase (27, 28). GFP-expressing cells transfected with Δ675 exhibited a significantly slower rate of accumulation of cells arrested in prophase compared with empty vector transfectants. Cells expressing wild-type hCdc5 displayed a slightly faster rise in prophase index compared with empty vector and a significantly accelerated increase compared with cells expressing the mutant. This result suggests that hCdc5 acts at least in part during G2 before the onset of prophase in mitosis. Taken together with the flow cytometry data (Fig. 5B), these findings strongly support a role for hCdc5 in the transcriptional regulation of G2 progression and mitotic entry.

hCdc5, S. pombe Cdc5, and other Cdc5-like proteins are putative transcription factors by virtue of their primary structures (19, 20, 29–31). In addition, we have shown that the carboxyl terminus of hCdc5 is capable of activating transcription in vitro (Fig. 4B). Genes regulated by Cdc5 family members, however, remain unknown. The Cdc2-cyclin B complex drives G2 progression in mammalian cells (3, 32). Inhibitory phosphorylation of Cdc2 by Wee1 kinase, and stimulatory dephosphorylation by Cdc25C phosphatase constitutes the major regulatory mechanism governing Cdc2-cyclin B activity (15, 16). S. pombe wee1 mutants and Cdc25 overexpressors display shortened G2 and reduced size (5). Since overexpression of hCdc5 in mammalian cells results in a similar phenotype, hCdc5 might be a transcriptional activator for Cdc25C or an inhibitor of Wee1 such as Nim1 (11, 12). Another formal possibility is that Cdc5 family members are regulated by and act as effectors of Cdc2 kinase. Although this is supported by the recent finding that a Xenopus laevis homolog of Cdc5 is an in vitro substrate for Cdc2 kinase (31), there is as yet no other evidence to suggest that transcriptional events regulated by Cdc2 are important for G2 progression and mitotic entry.

Characterization of hCdc5 provides a new tool for investigating and manipulating the mammalian cell cycle. Identification of genes regulated by hCdc5 will shed light on the precise mechanism by which Cdc5-like proteins regulate cell cycle progression, specifically G2/M. Moreover, inhibitors of hCdc5 function might prove useful for arresting cell proliferation in human neoplasms. Conversely, overexpression of hCdc5 in concert with other regulatory molecules may allow cellular regeneration of cardiac myocytes or other nondividing tissues.

Acknowledgments—We thank Xiao-qin Xu for technical assistance. Dr. Paul Dazin for assistance with flow cytometry analysis, Drs. Daniel Lerner and Stephen Hammes for thoughtful comments during the course of this work, and Drs. David Morgan, Patrick O’Farrell, and Henry Bourne for helpful discussion and critical reading of this manuscript.

REFERENCES

1. Murray, A., and Hunt, T. (1993) The Cell Cycle, Oxford University Press, Oxford

2. Nasmyth, K. (1996) Science 274, 1643–1645

3. Morgan, D. O. (1995) Nature 374, 131–134

4. Okayama, H., Nagata, A., Jinno, S., Murakami, H., Tanaka, K., and Nakashima, N. (1996) Adv. Cancer Res. 69, 17–62

5. Russell, P., and Nurse, P. (1987) Cell 49, 559–567

6. Lundgren, K., Walworth, N., Booser, R., Dembski, M., Kirschner, M., and Beach, D. (1991) Cell 64, 1111–1122

7. Fleig, U. N., and Gould, K. L. (1991) Semin. Cell Biol. 2, 195–204

8. Moreno, S., Nurse, P., and Russell, P. (1990) Nature 344, 549–552

9. Gautier, J., Solomon, M. J., Booser, R. N., Bazan, J. F., and Kirschner, M. W. (1991) Cell 67, 197–211

10. Coleman, T. R., Tang, Z., and Dunphy, W. G. (1993) Cell 72, 919–929

11. Parker, L. L., Walter, S. A., Young, P. G., and Piwnia-Worms, H. (1993) Nature 363, 736–738

12. Wu, L., and Russell, P. (1993) Nature 363, 738–741

13. Kinoshita, N., Yamano, H., Niwa, H., Yoshida, T., and Yamagida, M. (1993) Genes Dev. 7, 1059–1071

14. Shimizu, M., Ichikawa, K., Inoue, U., Nakamura, T., Nakajima, T., Nojima, H., Okayama, H., and Oda, K. (1995) Mol. Cell. Biol. 15, 2882–2892

15. Parker, L. L., and Piwnia-Worms, H. (1992) Science 257, 1955–1957

16. Lee, M. S., Ogg, S., Xu, M., Parker, L. L., Donoghue, D. J., Miller, J. L., and Piwnia-Worms, H. (1992) Mol. Biol. Cell 3, 73–84

17. Millar, J. B., Blewett, J., Garcez, L., Sudhu, K., Featherstone, C., and Russell, P. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 10500–10504

18. Nasmyth, K., and Nurse, P. (1981) EMBO J. 13, 471–483

19. Bernstein, H. S., and Coughlin, S. R. (1997) J. Biol. Chem. 272, 5833–5837

20. Iavarone, A., and Massague, J. (1997) EMBO J. 16, 471–483

21. Turner, K., and Gould, K. L. (1994) J. Biol. Chem. 269, 119–124

22. Harlow, E., and Lane, D. (1988) Antibodies: A Laboratory Manual, pp. 139–281, Cold Spring Harbor Laboratory, New York

23. Gossen, M., and Bujard, H. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 5457–5451

24. Reznitsky, D., Gossen, M., Bujard, H., and Reed, S. I. (1994) Mol. Cell. Biol. 14, 1669–1679
25. Nurse, P. (1975) Nature 256, 547–551
26. Quelle, D. E., Ashmun, R. A., Shurtleff, S. A., Kato, J. Y., Bar-Sagi, D., Roussel, M. F., and Sherr, C. J. (1993) Genes Dev. 7, 1559–1571
27. Kung, A. L., Sherwood, S. W., and Schimke, R. T. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 9553–9557
28. Krek, W., and DeCaprio, J. A. (1995) Methods Enzymol. 254, 114–124
29. Hirayama, T., and Shinozaki, K. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 13371–13376
30. Wilson, R., Ainscough, R., Anderson, K., Baynes, C., Berks, M., Bonfield, J., Burton, J., Connell, M., Copsey, T., Cooper, J., et al. (1994) Nature 368, 32–38
31. Stukenberg, P. T., Lustig, K. D., McGarry, T. J., King, R. W., Kuang, J., and Kirschner, M. W. (1997) Curr. Biol. 7, 338–348
32. King, R. W., Jackson, P. K., and Kirschner, M. W. (1994) Cell 79, 563–571
A Mammalian Homolog of Fission Yeast Cdc5 Regulates G2 Progression and Mitotic Entry
Harold S. Bernstein and Shaun R. Coughlin

J. Biol. Chem. 1998, 273:4666-4671.
doi: 10.1074/jbc.273.8.4666

Access the most updated version of this article at http://www.jbc.org/content/273/8/4666

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 30 references, 12 of which can be accessed free at http://www.jbc.org/content/273/8/4666.full.html#ref-list-1