AD_____

GRANT NUMBER DAMD17-98-1-8021

TITLE: Role of cdc25 Phosphatases in Cellular Immortalization

PRINCIPAL INVESTIGATOR: Konstantin I. Galaktionov, Ph.D.

CONTRACTING ORGANIZATION: Baylor College of Medicine
Houston, Texas 77030-3498

REPORT DATE: April 1999

TYPE OF REPORT: Annual

PREPARED FOR:
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

19991020 055

DTIC QUALITY INSPECTED 4

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503.

1. AGENCY USE ONLY *(Leave blank)*	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	April 1999	Annual (23 Mar 98 - 22 Mar 99)

4. TITLE AND SUBTITLE
Role of cdc25 Phosphatases in Cellular Immortalization

5. FUNDING NUMBERS
DAMD17-98-1-8021

6. AUTHOR(S)
Konstantin I. Galaktionov, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Baylor College of Medicine
Houston, Texas 77030-3498

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 words)*

The goal of this proposal is to study the potential role of tyrosine phosphorylation-dependent checkpoint and especially the cdc25 phosphatase family of cyclin dependent kinase (CDK) activators in cellular immortalization. We have shown that cdc25 phosphatases did in fact extend the normal mammary epithelial cells life span, but do not immortalize them. The effect was most prominent with cdc25A and cdc25B phosphatases. Cdc25C phosphatase had less effect on HMEC life span. Experiments with catalytically inactive cdc25A mutant show that phosphatase activity of cdc25A is essential for the life span extension. Our experiments shows that telomerase is not activated by cdc25A expression in normal human cells, suggesting that cdc25 most likely affect M1, but not M2 checkpoints.

14. SUBJECT TERMS	15. NUMBER OF PAGES
Breast Cancer , Cell Cycle	11
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

FOREWORD

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

N/A Where copyrighted material is quoted, permission has been obtained to use such material.

N/A Where material from documents designated for limited distribution is quoted, permission has been obtained to use the material.

N/A Citations of commercial organizations and trade names in this report do not constitute an official Department of Army endorsement or approval of the products or services of these organizations.

KFL In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and use of Laboratory Animals of the Institute of Laboratory Resources, national Research Council (NIH Publication No. 86-23, Revised 1985).

N/A For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law 45 CFR 46.

ICL In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

ICL In the conduct of research utilizing recombinant DNA, the investigator(s) adhered to the NIH Guidelines for Research Involving Recombinant DNA Molecules.

ICL In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.

PI - Signature

4/21/99
Date

Table of Contents

Front Cover	Page: 1
Report Documentation Page	Page: 2
Foreword	Page: 3
Table of Contents	Page: 4
Introduction	Page: 5
Body	Page: 6-8
Conclusion	Page: 8
References	Page: 8 - 11

Introduction

A variety of genetic abnormalities contribute to the transformation of normal cells into aggressively growing cancer cells. Among them, loss of the normal mechanisms of proliferation control is the common theme of most human malignancies.

The cell cycle in mammalian cells is driven by the family of evolutionary conserved cyclin-dependent kinases or CDKs (CDK1-8; Morgan, 1995). As cells progress in the cell cycle, CDKs are activated and promote further passage from one cell cycle phase to another by phosphorylation of critical substrates (reviewed in Draetta, 1990; Morgan, 1995). CDKs are inactive unless associated with the cyclin subunit. So far, eight different types of cyclins have been identified in human and other higher eukaryotes (cyclins A-H; reviewed in Hunter and Pines, 1994; Morgan, 1995; Sherr, 1996). Each CDK has a preferential cyclin subunit as a partner; however, some cyclins have the ability to associate with more than one CDK, creating a large number of cyclin/kinase complexes (Morgan, 1995; Sherr, 1996). Different cyclin-CDK complexes are activated at distinct points in the cell cycle (reviewed in Sherr, 1993,1996; Sherr and Roberts, 1995; Weinberg, 1995). In addition, mammalian CDKs are negatively regulated by the diverse family of cyclin-dependent kinase inhibitors or CKIs: p16ink4/MTS1, p15ink4b/MTS2, p21cip1/WAF1/SDI1(a transcriptional target of p53), p27kip1, p57kip2 and others (reviewed in Sherr and Roberts, 1995; Harper and Elledge, 1996). These molecules specifically target CDKs important for G1-S transition: CDK2, CDK4 and CDK6. Finally, CDKs are regulated by positive (CAK, or CDK activating kinase) or negative (wee1/mik1/myt1) phosphorylation. Phosphorylation by the wee1 family of kinases is reversed by the action of cdc25 phosphatases which have a unique specificity toward CDKs (reviewed in Millar and Russel, 1992; Draetta and Eckstein, 1997). The wee1/cdc25 checkpoint was originally described in fission yeast (Russell and Nurse, 1986) and later shown to represent a universal element of the cell cycle control (Russel, Moreno and Reed, 1989; Sadhu et al., 1990; Galaktionov and Beach, 1991). All CDKs (with the exception of cdk7) contain a conserved tyrosine and in the majority of CDKs, a neighboring threonine residue, targeted by the wee1 family of kinases (Gu et al., 1992; Parker et al., 1992; Mueller et al., 1995; Terada et al., 1995; Iavarone and Massague, 1997). Phosphorylation of a kinase on either or both of these residues renders it completely inactive. In human cells, there are three known cdk-activating phosphatases, cdc25A, B and C (Sadhu et al., 1990; Galaktionov and Beach, 1991).

Transformation of the normal human cell into an aggressively growing tumor cell typically requires a series of loss of function mutations in tumor supressor genes and gain of function mutations in oncogenes. Gain of function by gene amplification has been described for c-myc and cyclin D1 in several human tumors, including breast cancer. Cyclin D1 amplification is detected in 13% of breast cancers but more than 50% appear to overexpress the protein (Sherr, 1996). Targeted overexpression of cyclin D1 in mammary epithelial cells leads to hyperproliferation and eventual tumor formation (Wang et al., 1994).

We have shown that cdc25A and cdc25B phosphatases are overexpressed in a significant portion of primary breast cancer (Galaktionov et al., 1995; Galaktionov, Chen and Beach, 1996). This overexpression might be explained in part by the action of c-myc, which is amplified in 20-30% of breast cancers, and functions in part as a transcription factor for cdc25A (Galaktionov, Chen and Beach, ibid.). Overexpression of cdc25A and cdc25B is now described in other types of human cancer as well (Gasparotto et al., 1997). Ectopic expression of cdc25A in TGFß-sensitive breast cancer cell lines renders them insensitive to the inhibitory action of this cytokine (Iavarone and Massague, 1997). As a result, cells constitutively overexpressing cdc25 phosphatases might be partially or completely insensitive to the action of TGFß, especially when the p15ink4b/mts2 (CDK4/CDK6 inhibitor, activated by TGFb) locus is inactivated by deletion. Inability of cells overexpressing cdc25 to arrest in response to negative regulatory stimuli might contribute to abnormal cell proliferation and eventually tumor formation.

The broad aim of this proposal is to investigate how perturbation of the CDK tyrosine phosphorylation checkpoint might contribute to the oncogenic transformation process.

Body

Cdc25A and cell immortalization.

As proposed in Task1, we prepared recombinant retroviruses expressing each of the three human cdc25 genes: cdc25A, cdc25B and cdc25C (Sadhu *et al.*, 1990; Galaktionov *et al.*, 1991). We used pBABE-based retroviruses since these are stable retroviral vectors and we have had previous experience working with them (Galaktionov *et al.*, 1996). To show whether the phosphatase function of cdc25 is required, we also prepared a catalytically inactive cdc25A by substituting Ser for Cys in the catalytic center of cdc25A. The ensuing titers of these viruses exceed 10^5 per ml after transient transfection into BOSC or BING cells obtained from ATCC. Mammary epithelial cells (HMEC) were obtained at passage 12 from D. Beach, Cold Spring Harbor Laboratory. Using freshly prepared retroviral stocks we were able to achieve infection rates between 10 and 40 percent routinely. This enabled us to use large infected populations without relying on single cell clones in our analysis of the potential role of cdc25 phosphatases as immortalizing agents. Infected HMECs were adjusted to the same cell count after drug selection, and growth was continued with periodic cell culture split. HMECs have a finite life span and usually exhaust their proliferation ability at about passage 20. We infected HMECs at passage 14 and counted ensuing population doublings (PDL). Control HMECs underwent only 4-5 PDLs from the point of infection before they arrested with the typical senescent cell morphology. In contrast, cells expressing cdc25A and cdc25B phosphatases continued beyond this point for 6-8 additional population doublings to total of 12-13 (Figure 6).

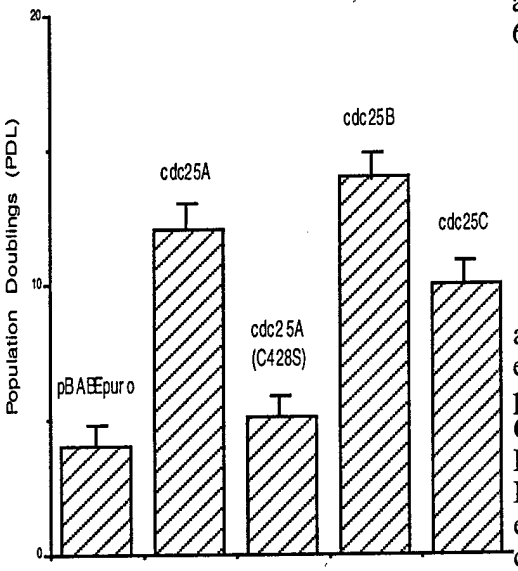

Figure 6. Life span extension by ectopic expression of cdc25 proteins. Cdc25 A, cdc25B and cdc25C, inactive cdc25A(C428S) proteins were expressed in normal human mammary epithelial cells infected with recombinant retroviruses.

We have performed these experiments three times and we are confident that cdc25 phosphatases did in fact extend the HMEC life span. The effect was most prominent with cdc25A and cdc25B phosphatases. Cdc25C phosphatase had less effect on HMEC life span. Experiments with catalytically inactive cdc25A(C482S, Figure 6) show that phosphatase activity of cdc25A is essential for the life span extension. Expression of catalytically inactive cdc25A does not cause any cell cycle arrest in HMEC, as they progress similarly to cells infected with the control retrovirus (pBABEpuro, Figure 6).

We had shown previously that a combination of oncogenic ras and cdc25A causes oncogenic transformation in rodent cells. These observations together with the experiments described here prompted us to investigate whether oncogenic rasV12 will affect the ability of cdc25A to cause life span extension in human HMEC cells. At first, we extended experiments of Serrano et al., 1997, who observed that oncogenic ras causes a paradoxical reaction in normal fibroblasts, resulting in a cell cycle arrest with some evidence of the premature cell senescence (Serrano et al., ibid.). The cell cycle arrest caused by the ectopic expression of rasV12 requires the wild type p53 protein and is mediated at least in part by the p19ARF, encoded by the alternative reading frame of the INK4a locus (reviewed in Sherr, 1998). HMEC displays a similar phenotype as they enter the cell cycle arrest a few days after infection with pBABEpuro retrovirus expressing rasV12. The senescence phenotype was confirmed by the cell morphology (flat enlarged cells with no cell division in more than 2 weeks) and staining for the senescence-specific b-galactosidase activity (Dimri et al., 1995).

Interestingly, co-expression of cdc25A or cdc25B together with rasV12 rescued HMEC cells from premature senescence caused by rasV12 (Figure 7).

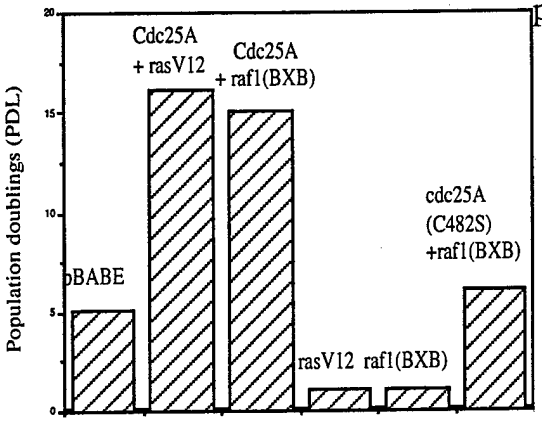

Figure 7. Life span extension by ectopic expression of cdc25A, in cooperation with rasV12 and raf1(BXB) proteins. Cdc25 A, phosphatase-"dead" cdc25A (C482S), rasV12, and raf1(BXB) proteins were expressed in normal human mammary epithelial cells infected with recombinant retroviruses. Note that rasV12 and raf1(BXB) cause a premature onset of senescence.

Because the raf/MEK/MAP kinase signaling cascade is a key effector of signaling from ras proteins, we examined the ability of raf1 kinase to affect HMEC cells. We observed that constitutively active raf1 kinase (BXB) was able to elicit cell cycle arrest and senescence (Figure 7). We wished to test whether cdc25A can reverse this arrest by co-expressing constitutively active raf1 and cdc25A. We observed that cell senescence caused by raf1 in HMEC cells was reversed by the action of cdc25A protein (Figure7). Cells expressing both cdc25A and raf1 progress further in their life span than cdc25A alone. Interestingly, our experiments also shown that the cdc25A reversal of the cell cycle arrest caused by raf1 does not require the phosphatase activity of cdc25A, but the subsequent bypass of the normal life span does (Figure 7). Experiments addressing further the mechanism of how cdc25A bypass the cell cycle arrest elicited in normal human cells by ras and raf1 are described in the Experimental Design Section.

Activation of telomerase activity is often associated with the so-called M2 checkpoint, positively correlating with cell immortalization. A product of the oncogenic papilloma virus, E6, has been shown to activate telomerase activity but does not immortalize normal human mammary epithelial cells (Klingelhutz et al., 1996). **As proposed in Task1**,we investigated whether this extension of the HMEC life span by cdc25 is associated with TGFβ resistance and activation of telomerase activity. To measure telomerase activity we used telomeric repeat amplification protocol (TRAP; Kim and Wu, 1997). As little as 1% telomerase-positive cells were detected by this method in a population of HMECs infected with retrovirus expressing the E6 gene product (Klingelhutz et al., ibid.). It has been shown recently that telomerase activity was not essential for establishment of immortal cell lines and growth of tumors in mice (Blasco et al., 1997), therefore we do not necessarily expect that presence or absence of telomerase activity will represent a conclusion for our experiments, but rather another measurable parameter in addition to PDLs. The recent literature (Bodnar et al., 1998), however, suggests that, in some types of human cells, ectopic expression of the catalytic telomerase subunit caused significant extension of the life span. It is possible that the contradiction between data obtained in mouse and human cells is due to species specificity and that telomerase activation is required for immortalization of the human cells. Our experiments shows that telomerase is not activated by cdc25a expression in normal human cells.

2. Role of wee1 kinases in establishment of the cellular senescence program

As proposed in task2, we performed low stringency PCR using degenerate oligonucleotides in order to clone putative wee1 homolog(s) with specificity toward cdk4 and cdk6 kinases. As a result, we obtain one sequence with significant sequence homology with known wee1 gene family members. However, this sequence seems to be expressed at very low levels in a majority of human tissues and cell lines and we are currently pursuing its cloning from several cDNA libraries. Low

representation of this clone so far prevented us from isolating a full length clone. **As proposed in task2**, we performed a two-hybrid screen using modified cdck4 protein. We are currently analysing cDMNAs obtained in that screen by sequencing. So far, we have not identified a putative wee1 family member. **As proposed in task2**, we also started an attempt of biochemical purification of wee1 kinase, specific for cdk4. To that extent we have prepared a cellular extract from Hela cells and are currently characterising fractions obtained by anion exchange chromatography on FPLC trying first to identify the appropriate activity. This is a long term project and we don't expect a rapid progress here.

CONCLUSIONS

1. Cdc25 phosphatases expand the life span of normal mammary epithelial cells (HMEC).
2. Cdc25 phoshatases did not cause complete immortalization of HMEC
3. Phosphatase activity of cdc25A is required for the observed phenotype.
4. Cdc25 did not cause activation of telomerase in HMEC.
5. Cdc25A cooperated with RasVal12 to further expand their lifespan and reverse "senescent" phenotype caused by RasV12 in HMEC.

REFERENCES

Alcorta, D.A., Xiong, Y., Phelps, D., Hannon, G., Beach, D. and Barrett, J.C. (1996) Involvement of the cyclin-dependent kinase inhibitor p16 (INK4a) in replicative senescence of normal human fibroblasts. Proc. Natl. Acad. Sci.U S A 93 (24): 13742-13747.

Blasco, M.A., Lee, H.W., Hande, M.P., Samper, E., Lansdorp, P.M., DePinho, R.A. and Greider, C.W. (1997). Telomere shortening and tumor formation by mouse cells lacking telomerase RNA. Cell 91: 25-34.

Bodnar, A.G., Ouelette, M., Frolkis, M., Holt, S.E., Chiu, C.-P., Morin, G.B., Harley, C.B., Shay, J.W., Lichtsteiner, S.B., Wright, W.E. (1998). Extension of life-span by introduction of telomerase into normal human cells. Science 278: 349-355.

Booher, R.N., Holman, P.S. and Fattaey, A. (1997). Human Myt1 is a cell cycle- regulated kinase that inhibits Cdc2 but not Cdk2 activity. J Biol Chem 272: 22300-22306.

Dimri, G.P., Lee, X., Basile, G., Acosta, M., Scott, G., Roskelley, C., Medrano, E.E., Linskens, M., Rubeli, I., Pereira-Smith, O., Peacocke, M., and Campisi, J. (1995). A biomarker that identifies senescent human cells in culture and in aging skin in vivo. Proc Natl Acad Sci USA 92: 9363-9367.

Dowell, S.J., Romanowski, P. and Diffley, J.F. (1994). Interaction of Dbf4, the Cdc7 protein kinase regulatory subunit, with yeast replication origins in vivo. Science 265: 1243-1246.

Draetta, G. (1990). Cell cycle control in eukaryotes; molecular mechanisms of cdc2 activation. Trends. Biol.Sci. 15: 378-383.

Draetta, G. and Eckstein, J. (1997). Cdc25 protein phosphatases in cell proliferation. Biochim. Biophys. Acta 1332: M53-M63.

el-Deiry, W.S., Tokino, T., Velculescu, V.E., Levy, D.B., Parsons, R., Trent, J.M., Lin, D., Mercer, W.E., Kinzler, K.W. and Vogelstein, B. (1993). WAF1, a potential mediator of p53 tumor suppression. Cell 75: 817-825.

Fisher, R.P, and Morgan, D.O. (1994). A novel cyclin associates with MO15/CDK7 to form the CDK-activating kinase. Cell 78:713-724.

Furnari, B., Rhind, N. and Russell, P. (1997). Cdc25 mitotic inducer targeted by chk1 DNA damage checkpoint kinase. Science 277: 1495-1497.

Gabrielli, B.G., De Souza, C.P., Tonks, I.D., Clark, J.M., Hayward, N.K. and Ellem, K.A. (1996). Cytoplasmic accumulation of cdc25B phosphatase in mitosis triggers centrosomal microtubule nucleation in HeLa cells. J Cell Sci 109: 1081-1093.

Galaktionov, K. and Beach, D. (1991). Specific activation of cdc25 tyrosine phosphatases by B-

type cyclins: evidence for multiple roles of mitotic cyclins. Cell 67:1181-1194.
Galaktionov, K., Lee, A.K., Eckstein, J., Draetta, G., Meckler, J., Loda, M. And Beach, D. (1995). CDC25 phosphatases as potential human oncogenes. Science 269: 1575-1577.
Galaktionov, K., Chen, X. and Beach, D. (1996). Cdc25 cell-cycle phosphatase as a target of c-myc. Nature 382: 511-517.
Gu, Y., Rosenblatt, J. and Morgan, D.O. (1992). Cell cycle regulation of cdk2 activity by phosphorylation of Thr160 and Tyr15. EMBO J. 11: 3995-4005.
Guan, K.L., Jenkins, C.W., Li, Y., Nichols, M.A., Wu, X., O'Keefe, C.L., Matera, A.G. and Xiong, Y. (1994). Growth suppression by p18, a p16INK4/MTS1- and p14INK4B/MTS2-related CDK6 inhibitor, correlates with wild-type pRb function. Genes Dev Dec 8:2939-2952.
Gudkov, A.V., Kazarov, A.R., Thimmapaya, R., Axenovich, S.A., Mazo, I.A. and Roninson, I.B. (1994). Cloning mammalian genes by expression selection of genetic suppressor elements: association of kinesin with drug resistance and cell immortalization. Proc Natl Acad Sci USA 91: 3744-3748.
Hannon, G.J., Demetrick, D. and Beach, D. (1993). Isolation of the Rb-related p130 through its interaction with CDK2 and cyclins. Genes Dev 7: 2378-2391.
Harper, J.W. and Elledge, S.J. (1996). Cdk inhibitors in development and cancer. Curr. Opin. Genet. Dev. 6: 56-64.
Hirai, H., Roussel, M.F., Kato, J.Y., Ashmun, R.A. and Sherr, C.J. (1995). Novel INK4 proteins, p19 and p18, are specific inhibitors of the cyclin D-dependent kinases CDK4 and CDK6. Mol. Cell Biol. 15: 2672-2681.
Hoffmann, I., Draetta, G. and Karsenti, E. (1994). Activation of the phosphatase activity of human cdc25A by a cdk2-cyclinE dependent phosphorylation at the G1/S transition. EMBO J 13: 4302-4310.
Hunter T, Pines J (1994). Cyclins and cancer. II: Cyclin D and CDK inhibitors come of age. Cell 79: 573-582.
Iavarone, A. and Massague, J. (1997). Repression of the CDK activator Cdc25A and cell-cycle arrest by cytokine TGF-beta in cells lacking the CDK inhibitor p15. Nature 387: 417-422.
James, P., Halladay, J. and Craig, E.A. (1996). Genomic libraries and a host strain designed for highly efficient two-hybrid selection in yeast. Genetics 144:1425-1436.
Jinno, S., Suto,K., Nagata,A., Igarashi,M., Kanaoka,Y., Nojima,H. and Okayama,H. (1994).Cdc25A is a novel phosphatase functioning early in the cell cycle. EMBO J. 13: 1549-1556.
Kaldis, P., Sutton, A., Solomon, M.J. (1996). The Cdk-activating kinase (CAK) from budding yeast. Cell 86 (4): 553-564.
Kim, N.W. and Wu, F. (1997). Advances in quantification and characterization of telomerase activity bythe telomeric repeat amplification protocol (TRAP). Nucleic Acids Res 25: 2595-2597.
Klingelhutz, A.J., Foster, S.A. and McDougall, J.K. (1996). Telomerase activation by the E6 gene product of human papillomavirus type16. Nature 380: 79-82.
Kumagai, A. and Dunphy, W.G. (1992). Regulation of the cdc25 protein during the cell cycle in Xenopus extracts. Cell 70: 139-151.
Kumagai, A. and Dunphy, W.G. (1996). Purification and molecular cloning of Plx1, a Cdc25 regulatory kinasefrom Xenopus egg extracts. Science 273: 1377-1380.
Lucibello, F.C., Truss, M., Zwicker, J., Ehlert, F., Beato, M. and Muller, R. (1995). Periodic cdc25C transcription is mediated by a novel cell cycle-regulated repressor element (CDE). EMBO J 14: 132-142.
Makela, T.P., Tassan, J.-P., Nigg, E.A., Frutiger, S., Hughes, G. and Weinberg, R.A. (1994). A cyclin associated with the cdk-activating kinase MO15. Nature 371:254-257.
Millar, J.B. and Russell, P. (1992). The cdc25 M-phase inducer: an unconventional protein phosphatase. Cell 68: 407-410.
Morgan, D.O. (1995). Principles of CDK regulation. Nature 374: 131-134.
Morgenstern, J.P. and Land, H. (1990). Advanced mammalian gene transfer: high titre

retroviral vectors with multiple drug selection markers and a complementary helper-free packaging cell line. Nucleic Acids Res. 18: 3587-3596.
Mueller, P.R., Coleman, T.R., Kumagai,A. and Dunphy, W.G. (1995). Myt1: a membrane-associated inhibitory kinase that phosphorylates Cdc2 on both threonine-14 and tyrosine-15. Science 270 (5233): 86-90.
Nagata, A., Igarashi, M., Jinno, S., Suto, K. and Okayama, H. (1991). An additional homolog of the fission yeast cdc25+ gene occurs in humans and is highly expressed in some cancer cells. New Biol. 3:959-968.
Noda, A., Ning, Y., Venable, S.F., Pereira-Smith, O.M. and Smith, J.R. (1994). Cloning of senescent cell derived inhibitors of DNA synthesis using an expression screen. Exp Cell Res 211: 90-98.
Parker, L.L., Atherton-Fessler, S. and Piwnica-Worms, H. (1992). p107wee1 is a dual-specificity kinase that phosphorylates p34cdc2 on tyrosine 15. Proc Natl Acad Sci U S A 89: 2917-2921.
Peng, C.Y., Graves, P.R., Thoma, R.S., Wu, Z., Shaw, A.S. and Piwnica-Worms, H. (1997). Mitotic and G2 checkpoint control: regulation of 14-3-3 protein binding by phosphorylation of Cdc25C on serine-216. Science 277: 1501-1505.
Polyak, K., Lee, M., Erdjument-Bromage, H., Koff, A., Roberts, J.M., Tempst, P. and Massague, J. (1994). Cloning of p27Kip1, a cyclin-dependent kinase inhibitor and a potential mediator of extracellular antimitogenic signals. Cell 78: 59-66.
Russell, P., Nurse, P. (1986). cdc25+ functions as an inducer in the mitotic control of fission yeast. Cell 45: 145-153.
Russell, P., Moreno,S. and Reed, S.I. (1989). Conservation of mitotic controls in fission and budding yeasts. Cell 57: 295-303.
Sadhu, K., Reed, S.I., Richardson, H. and Russell, P. (1990). Human homolog of fission yeast cdc25 mitotic inducer is predominantly expressed in G2. Proc. Natl. Acad. Sci. U S A 87: 5139-5143.
Saha, P., Eichbaum, Q., Silberman, E.D., Mayer, B.J. and Dutta, A. (1997). p21CIP1 and Cdc25A: competition between an inhibitor and an activatorof cyclin- dependent kinases. Mol Cell Biol 17: 4338-4345.
Sanchez, Y., Wong, C., Thoma, R.S., Richman, R., Wu, Z., Piwnica-Worms, H. and Elledge, S.J. (1997). Conservation of the Chk1 checkpoint pathway in mammals: linkage of DNA damage to Cdk regulation through Cdc25. Science 277: 1497-1501.
Serrano, M., Hannon, G.J. and Beach, D. (1993). A new regulatory motif in cell-cycle control causing specific inhibition of cyclin D/CDK4. Nature 366:704-707.
Serrano, M., Lin , A.W., McCurrach, M.E., Beach, D and Lowe,S.W. (1997). Oncogenic ras provokes premature cell senescence associated with accumulation of p53 and p16INK4a. Cell 88: 593-602.
Sherr, C.J. (1993). Mammalian G1 cyclins. Cell 73: 1059-1065.
Sherr, C.J. (1996). Cancer cell cycles. Science 274:1672-1677.
Sherr, C.J. (1998). Tumor surveillance via the ARF-p53 pathway. Genes Dev. 12:2984- 2991.
Sherr, C.J. and Roberts, J.M. (1995). Inhibitors of mammalian G1 cyclin-dependent kinases. Genes Dev. 9: 1149-1163.
Simmons, D.L., Neel, B.G., Stevens, R., Evett, G. and Erikson, R.L. (1992). Identification of an early growth-response gene encoding a novel putative protein kinase. Mol Cell Biol 12: 4164-4169.
Spitkovsky, D., Jansen-Durr, P., Karsenti, E. and Hoffman, I. (1996). S-phase induction by adenovirus E1A requires activation of cdc25a tyrosine phosphatase. Oncogene 12: 2549-2554.
Terada, Y., Tatsuka, M., Jinno, S., and Okayama, H. (1995). Requirement for tyrosine phosphorylation of Cdk4 in G1 arrest induced by ultraviolet irradiation. Nature 376:358-362.
Walworth, N.C. and Bernards, R. (1996). Rad-dependent response of the chk1-encoded protein kinase at the DNA damage checkpoint. Science 271: 353-356.

Watanabe, N., Broome, M. and Hunter,T. (1995). Regulation of the human WEE1Hu CDK tyrosine 15-kinase during the cell cycle. EMBO J. 14: 1878-1891.

Weinberg, R.A. (1995). The retinoblastoma protein and cell cycle control. Cell 81: 323- 330.

Wright, W.E., Pereira-Smith, O.M. and Shay, J.W. (1989). Reversible cellular senescence: implications for immortalization of normal human diploid fibroblasts. Mol Cell Biol 9: 3088-3092.