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G_1 control in mammalian cells

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SUMMARY

Cyclin-dependent kinases (Cdks) control the major cell cycle transitions in eukaryotic cells. On the basis of a variety of experiments where cyclin function either is impaired or enhanced, D-type cyclins as well as cyclins E and A have been linked to G_1 and G_1/S phase roles in mammalian cells. We therefore sought to determine if agents that block the G_1/S phase transition do so at the level of regulating the Cdk activities associated with these cyclins. A variety of conditions that lead to G_1 arrest were found to correlate with accumulation of G_1-specific Cdk inhibitors, including treatment of fibroblasts with ionizing radiation, treatment of epithelial cells with TGF-β, treatment of HeLa cells with the drug lovastatin, and removal of essential growth factors from a variety of different cell types. Mechanistically, inhibition of Cdks was found to involve the stoichiometric binding of Cdk inhibitor proteins. $p21^{Waf1/Cip1}$ was associated with DNA damage induced arrest while $p27^{Kip1}$/$p28^{Ick1}$ accumulated under a variety of antiproliferative conditions.

Key words: cell cycle, G_1, cyclin, Cdk

INTRODUCTION

Understanding of the eukaryotic cell cycle has been revolutionized by the discovery that all of the major regulatory points are controlled by a class of protein kinases known as cyclin-dependent kinases, or Cdks (for reviews see Sherr, 1993; Reed, 1992; Pines and Hunter, 1991). The functional paradigm of these kinases is that they are composed of a catalytic subunit that has no intrinsic activity and a requisite positive regulatory subunit known as a cyclin. In simple eukaryotic organisms such as yeast, a single Cdk catalytic subunit interacts with numerous different classes of cyclin to mediate diverse cell cycle phase transitions. In mammalian cells, the regulatory environment is complicated by the presence of multiple Cdks (Meyerson et al., 1992) as well as cyclins (Xiong and Beach, 1991; Lew and Reed, 1992). It has been our goal to elucidate the roles of these kinases and cyclins in the regulation of the G_1 to S phase transition.

RESULTS AND DISCUSSION

G_1 cyclins

A number of lines of evidence implicate three types of cyclin in G_1 and S phase functions. Cyclins D1 and E were identified on the basis of their ability to rescue a deficiency of G_1 cyclin function in the simple eukaryote yeast (Lew et al., 1991; Xiong et al., 1991; Koff et al., 1991). At the same time, the cyclin D1 cDNA was isolated on the basis of the growth factor inducibility of the corresponding mRNA (Matsushime et al., 1991) and because of its location at a chromosomal breakpoint associated with malignancy (Motokura et al., 1991; Withers et al., 1991). After the identification of cyclin D1, two other closely related cyclins, D2 and D3, were identified on the basis of their homology to cyclin D1 (Matsushime et al., 1991). Patterns of expression suggested that cyclins D and E were associated with G_1 and/or G_1/S phase functions (Dulic et al., 1992; Koff et al., 1992; Baldin et al., 1993; Won et al., 1992). Finally, cyclin A, shown to accumulate beginning at the G_1/S phase boundary, was suggested to have a potential role during S phase (Pines and Hunter, 1990).

Several lines of evidence confirm G_1 or G_1/S phase roles for these three classes of cyclin. Antibody microinjection experiments using IgGs specific for cyclins A and D1 have demonstrated that these cyclins are required for initiation or progression through S phase (Girard et al., 1991; Pagano et al., 1992; Zindy et al., 1992; Baldin et al., 1993; Quelle et al., 1993). Similar experiments using cyclin E-specific antibodies have been unsuccessful, possibly for technical reasons (G. Mondesert and S. Reed, unpublished). However, cyclin E has been shown to be essential for entry into S phase in *Drosophila* (Knoblich et al., 1994), suggesting an essential S phase initiation function in mammalian cells as well. Conversely, constitutive expression of these three cyclins can lead to premature entry into S phase, suggesting that they control functions that are rate-limiting for passage through G_1 into S phase (Ohtsubo and Roberts, 1993; Quelle et al., 1993; Resnitzky et al., 1994; D. Resnitzky and S. Reed, unpublished; Table 1). However, expression of no cyclin alone appears to be able to advance the G_1/S phase to the degree that G_1 can be eliminated. This suggests either that several individual rate-limiting transitions within G_1 are controlled by different cyclins or that compartments exist within the G_1 interval that cannot be influenced by the action of cyclin-dependent kinases.

Table 1. Effect of cyclin E and cyclin D1 on asynchronous cells versus cells emerging from quiescence

Clone	% G_1 in asynchronous populations		Length of G_1 in asynchronous populations (h)		Decrease in length of G_1 in asynchronous populations (h)	Decrease in length of G_0/G_1 after serum starvation/ stimulation (h)
	+tet	−tet	+tet	−tet		
D5	48.4	41.8	6.78	5.85	0.93±0.17	4.7±0.7
D3	44.1	39.9	6.17	5.58	0.53±0.11	3.1±1.1
E2	52.2	39.9	7.31	5.59	1.72±0.04	2.5±0.9
E19	49.2	36.9	6.89	5.17	1.72±0.18	1.7±0.7

The percentage of G_1 cells in asynchronous populations was determined by flow cytometric analysis and was used with the population doubling time to determine the length of the G_1 phase. The relative lengths of G_0/G_1 in populations emerging from quiescence was determined by direct measurement using flow cytometric analysis to monitor entry into S phase. In all experiments, comparisons were based on cultures growing in the presence of tetracycline (cyclins repressed) versus in the absence of tetracycline (cyclins repressed) (Resnitzky et al., 1994). Clones D5 and D3 are Rat1 fibroblasts expressing human cyclin D1 and clones E2 and E19 express human cyclin E.

It is of interest to note that whereas constitutive expression of cyclins D1 and E have a more profound effect on advance of S phase in cells emerging from quiescence as compared to cycling cells, expression of cyclin E confers an equivalent S phase advance in either circumstance, suggesting participation in distinct pathways (Resnitzky et al., 1994; Table 1).

Finally, with the demonstration that mammalian cells contain a number of different Cdk catalytic subunits, it was important to establish which combinations of G_1 cyclins and catalytic subunits contribute to the activities described above. Although a large number of combinatorial possibilities have been detected, the biologically critical complexes for G_1/S-phase functions appear to be cyclins A and E combined with catalytic subunit Cdk2 (Pines and Hunter, 1990; Dulic et al., 1992; Koff et al., 1992) and D type cyclins combined with Cdk4 (Matsushime et al., 1992; see Fig. 1).

Targets of cyclin-dependent kinase action

Of all the potential targets for the G_1 cyclin-dependent kinases, the retinoblastoma susceptibility gene product, pRb is the one most often invoked to explain regulation of the G_1/S phase transition. It is known that pRb inhibits cell cycle progression when in a hypophosphorylated form and that phosphorylation reverses these inhibitory functions (Buchkovich et al., 1989; Chen et al., 1989; DeCaprio et al., 1992; Ludlow et al., 1993; Mihara et al., 1989). A mounting body of evidence suggests that the inhibitory function of pRb is based on the ability of hypophosphorylated pRb to sequester some members of a family of essential transcription factors known together as E2F (Helin et al., 1992; Kaelin et al., 1992; Nevins, 1992). Phosphorylation of pRb is thought to lead to the release of transcription-competent E2F. Furthermore, the in vivo phosphorylation sites on pRb correspond to Cdk consensus phosphorylation sites and a variety of Cdks have been shown to be capable of phosphorylating pRb in vitro (Lees et al., 1991; Lin et al., 1991; Kato et al., 1993). The most compelling evidence for an in vivo role for Cdks in pRb phosphorylation comes from experiments in SAOS-2 osteosarcoma cells where ectopic expression of pRb caused G_1 arrest but co-expression of pRb with either cyclin D, E or A did not (Hinds et al., 1992; Ewen et al., 1993). It could be shown that, concomitant with the release from G_1 arrest, pRb became hyperphosphorylated. These data suggest that all of the proposed G_1 and G_1/S phase Cdk activities have the capacity to phosphorylate pRb directly or to stimulate its phosphorylation by other protein kinases in vivo. These experiments have the caveat, however, that both pRb and cyclins are highly overexpressed, perhaps imbuing them with abnormal properties. Therefore the issue of which Cdks (cyclins and catalytic subunits) actually contribute to pRb phosphorylation under normal cellular growth circumstances remains to be resolved. It is noteworthy that D-type cyclins contain motifs that allow them to bind directly to pRb, presumably facilitating the direction of kinase activity toward this potential substrate (Ewen et al., 1993; Dowdy et al., 1993).

Several other proteins with Rb-like physical proterties have been identified. p107 and p130 have structural homology with pRb, can bind viral oncoproteins, as can pRb, and can bind E2F as well (Ewen et al., 1991; Hannon et al., 1993). However the relationship of these proteins to cell cycle control is less well understood than that of pRb. High levels of expression of p107 in some cell types confer G_1 arrest, suggesting an inhibitory role similar to that of pRb (Zhu et al., 1993). The relationship of cell cycle control to sequestration of E2F is less clear. Although p107 and p130 bind E2F, it appears that different isoforms are involved relative to pRb (Lees et al., 1993). The roles of these isoforms have not yet been elucidated nor has the relationship between phosphorylation of p107 and p130 and release of E2F. However, it has been shown that cyclin

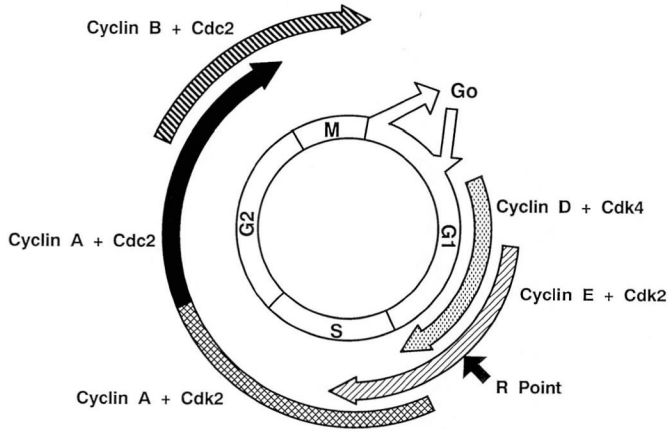

Fig. 1. The roles and temporal participation of various cyclins and cyclin-dependent kinases in the mammalian cell cycle.

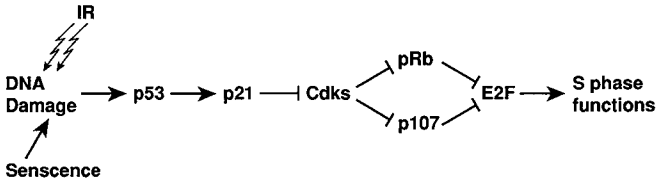

Fig. 2. The functions of p53 and p21 in mediating cell cycle control in response to DNA damage. DNA damage incurred from external sources such as ionizing radiation (IR) or internal sources, such as the transition to senescence, leads to elevation of p53 levels. p53 promotes elevated expression of p21, which in turn inhibits G_1 Cdks. This ultimately has the effect of inhibiting functions essential for S phase, notably the the release of E2F isoforms from antagonists such as pRb and p107.

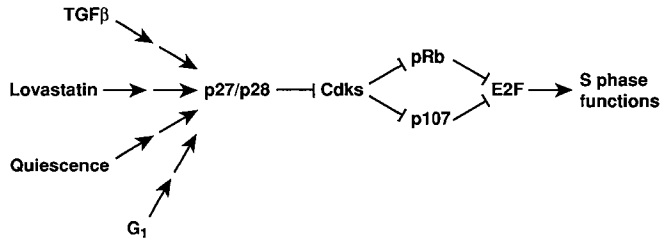

Fig. 3. Mediation of cell cycle control by p27/p28. A variety of conditions, including response to TGFβ (epithelial cells), treatment with lovastatin, starvation for serum or contact inhibition, and passage through the G_1 phase of the cell cycle, lead to accumulation of the Cdk inhibitor $p27^{Kip1}/p28^{Ick1}$. As with p21, this is likely to prevent the release of the essential S phase transcription factor E2F.

E/Cdk2 is included in p107/E2F complexes in late G_1 and early S phase whereas cyclin A/Cdk2 is in similar complexes during S phase and G_2 (Lees et al., 1992). This suggests a regulatory role for cyclin E and cyclin A in regulation of p107 functions. If p107 functions analogously to pRb, then cyclin E/Cdk2 may control release of E2F isoforms associated with p107 by direct phosphorylation of p107. Other potential targets of G_1 Cdks are proteins directly involved in initiation of DNA replication, such as origin binding proteins. However, these remain to be identified before this idea can be explored.

The Role(s) of Cdk inhibitors

In budding yeast, developmental control of the cell cycle occurs at G_1 and is implemented at the level of controlling G_1-specific Cdks (Reed, 1992). Therefore, investigation of G_1 control in mammalian cells has focused on G_1 Cdks, as well (Sherr, 1993). Several model systems have been explored including the effects of DNA damage (Dulic et al., 1994), negative growth factors (Koff et al., 1993; Polyak et al., 1994; Slingerland et al., 1994), antiproliferative drugs (Hengst et al., 1994) and growth factor deprivation (Dulic et al., 1994; Hengst et al., 1994; Polyak et al., 1994; Slingerland et al., 1994). A common theme has emerged, namely that G_1 control in response to a variety of signals is mediated at the level of proteins that inhibit the kinase activities of Cdks via stoichiometric binding (Dulic et al., 1994; Hengst et al., 1994; Polyak et al., 1994; Slingerland et al., 1994).

When human diploid fibroblasts are synchronized by growth to confluence followed by replating, they can be prevented from entering S phase by high doses of ionizing radiation such as γ-rays (Little and Nagasawa, 1985). Unirradiated cells enter S phase at approximately 18 hours after release from contact inhibition. Cells blocked in G_1 were found to accumulate normal levels of cyclin E and D1 and to form normal levels of cyclin E/Cdk2 and cyclin D1/Cdk4 complexes. However, these complexes were found not to be active (Dulic et al., 1994). It was shown that cyclin/Cdk complexes under these conditions contained a heat stable Cdk inhibitory protein whose presence was dependent on the wild-type status of the tumor suppressor p53. Further investigation indicated that this inhibitor corresponded to $p21^{Waf1/Cip1}$ (El-Deiry et al., 1993; Harper et al., 1993; Xiong et al., 1993; Gu et al., 1993), shown to be a p53-inducible Cdk inhibitor (Dulic et al., 1994). These data establish a p53-dependent signal transduction pathway linking DNA damage to cell cycle control (Fig. 2). The loss of genome stability in response to negation of p53 function is likely to arise from the uncoupling of cell cycle control from DNA damage responses. This is likely to be an important modality in the transition to malignancy. Similar data have been obtained for senescent fibroblasts (Dulic et al., 1993) where DNA damage signals have been invoked to explain the block to proliferation.

The response of epithelial cells to epithelial antiproliferative agent, TGF-β, is phenomenologically similar to the response to DNA damage. In human breast epithelial cells presynchronized by growth factor depletion and refeeding, and then treated with TGF-β, cyclin E accumulates on schedule, associates with Cdk2, but cyclin/Cdk complexes are inactive (Slingerland et al., 1994). Similarly, in the situation with ionizing radiation, a heat stable Cdk inhibitor could be detected in lysates from TGF-β treated cells (Slingerland et al., 1994; E. Bailly and S. Reed, unpublished). However it is unlikely that this inhibitor is $p21^{Waf1/Cip1}$. Similar studies on mink lung epithelial cells suggest that the inhibitor induced by treatment with TGF-β is a 27/28 kDa protein that is distinct from p21 (Polyak et al., 1994). This protein has been alternately designated $p27^{Kip1}$ and $p28^{Ick1}$.

p27/p28 accumulates under a number of conditions associated with G_1 arrest of the cell cycle (Fig. 3). HeLa cells treated with the drug lovastatin, which confers G_1 arrest, accumulate p27/p28 in inactive cyclin/Cdk complexes (Hengst et al., 1994). Since p27/p28 is heat stable, it can be released from these complexes by boiling. p27/p28 also accumulates in fibroblasts and epithelial cells deprived of essential growth factors (Hengst et al., 1994). It has also been shown to fluctuate through the cell cycle in proliferating cells, with maximal levels in mid-G_1 (Hengst et al., 1994). Finally, like p21, it has been shown to have the ability to bind and inhibit a broad spectrum of Cdks (Hengst et al., 1994). These data suggest that a variety of conditions that confer G_1 arrest in mammalian cells lead to the production or maintenance of a Cdk inhibitor, p27/p28 that has the capacity to arrest the cell cycle in G_1. In addition, this inhibitor fluctuates through the cell cycle, presumably to prevent premature activation of Cdks during the G_1 interval. Finally, p27/p28 and p21 appear to play complementary roles in cell cycle control mediating the antiproliferative responses associated with distinct signals. In particular, p21 seems to be associated with responses to DNA damage, whereas p27/p28 appears to mediate a more general antiproliferative response.

In summary, the primary motif of cell cycle regulation in G_1 appears to be through the action of cell cycle inhibitory proteins that bind to and inhibit Cdks by a stoichiometric mechanism. This is distinct from control of the G_2/M phase transition, which is mediated primarily at the level of negative phosphorylation of the Cdk1 catalytic subunit (Gould and Nurse, 1989). One possible reason for the prevalence of broad spectrum Cdk inhibitors in G_1 control is that it would allow coordinate control of several distinct Cdks thought to collaborate in progression through G_1 to S phase. It is not yet clear whether p21 and p27/p28 define all of the Cdk inhibitors involved in cell cycle control or whether they are only the first members of a larger family to be identified.

REFERENCES

Baldin, V., Lukas, J., Marcote, M. J., Pagano, M. and Draetta, G. (1993). Cyclin D1 is a nuclear protein required for cell cycle progression in G_1. *Genes Dev.* **7**, 812-821.

Buchkovich, K., Duffy, L. A. and Harlow, E. (1989). The retinoblastoma protein is phosphorylated during specific phases of the cell cycle. *Cell* **58**, 1097-1105.

Chen, P.-L., Scully, P., Shew, J.-Y., Wang, J. Y. J. and Lee, W.-H. (1989). Phosphorlylation of the retinoblastoma gene product is modulated during the cell cycle and cellular differentiation. *Cell* **58**, 1193-1198.

DeCaprio, J. A., Furukawa, Y., Ajchenbaum, F., Griffin, J. D. and Livingston, D. (1992). The retionblastoma-susceptibility gene product becomes phosphorylated in multiple stages during cell cycle entry and progression. *Proc. Nat. Acad. Sci. USA* **89**, 1795-1798.

Dowdy, S. F., Hinds, P. W., Louie, K., Reed, S. I., Arnold, A. and Weinberg, R. A. (1993). Physical interaction of the retinoblastoma protein with human D cyclins. *Cell* **73**, 499-511.

Dulic, V., Lees, E. and Reed, S. I. (1992). Association of human cyclin E with a periodic G_1-S phase protein kinase. *Science* **257**, 1958-1961.

Dulic, V., Drullinger, L. F., Lees, E., Reed, S. I. and Stein, G. H. (1993). Alternate regulation of G_1 cyclins in senescent human fibroblasts: Accumulation of inactive cyclin D1/Cdk2 and cyclin E/Cdk2 complexes. *Proc. Nat. Acad. Sci. USA* **90**, 11034-11038.

Dulic, V., Kaufmann, W. K., Wilson, S. J., Tlsty, T. D., Lees, E., Harper, J. W., Elledge, S. J. and Reed, S. I. (1994). p53-dependent inhibition of cyclin-dependent kinase activities in human fibroblasts during radiation-induced G_1 arrest. *Cell* **76**, 1013-1023.

El-Deiry, W. S., Tokino, R., 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.

Ewen, M. E., Xing, Y., Lawrence, J. B. and Livingston, D. M. (1991). Molecular cloning, chromosomal mapping, and expression of the cDNA for p107, a retinoblastoma gene product-related protein. *Cell* **66**, 1155-1164.

Ewen, M. E., Sluss, H. K., Sherr, C. J., Matsushime, H., Kato, J.-Y. and Livingston, D. M. (1993). Functional interactions of the retinoblastoma protein with mammalian D-type cyclins. *Cell* **73**, 487-497.

Girard, F., Strausfeld, U., Fernandez, A. and Lamb, N. (1991). Cyclin A is required for the onset of DNA replication in mammalian fibroblasts. *Cell* **67**, 1169-1179.

Gould, K. and Nurse, P. (1989). Tyrosine phosphorylation of the fission yeast $cdc2^+$ protein kinase regulates entry into mitosis. *Nature* **342**, 39-45.

Gu, Y., Turck, C. W. and Morgan, D. O. (1993). Inhibition of Cdk2 activity in vivo by an associated 20K regulatory subunit. *Nature* **366**, 707-710.

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., Adami, G. R., Wei, N., Kayomarsi, K. and Elledge, S. J. (1993). The p21-Cdk-interacting protein, Cip1 is a potent inhibitor of G_1 cyclin-dependent kinases. *Cell* **75**, 805-816.

Helin, K., Lees, J. A., Vidal, M., Dyson, N., Harlow, E. and Fattaey, A. (1992). A cDNA encoding a pRB-binding protein with properties of the transcription factor E2F. *Cell* **70**, 337-350.

Hengst, L., Dulic, V., Slingerland, J. M., Lees, E. and Reed, S. I. (1994). A cell cycle regulated inhibitor of cyclin dependent kinases. *Proc. Nat. Acad. Sci. USA* **91**, 5291-5295.

Hinds, P. W., Mittnacht, S., Dulic, V., Arnold, A., Reed, S. I. and Weinberg, R. A. (1992). Regulation of retinoblastoma protein functions by ectopic expression of human cyclins. *Cell* **70**, 993-1006.

Kaelin, W. G., Jr, Krek, W., Sellers, W. R., DeCaprio, J. A., Ajchenbaum, F., Fuchs, C. S., Chittenden, T., Li, Y., Farnham, P. J., Blanar, M. A., Livingston, D. M. and Flemington E. K. (1992). Expression cloning of a cDNA encoding a retinoblastoma-binding protein with E2F-like properties. *Cell* **70**, 351-364.

Kato, J.-Y., Matsushime, H., Hiebert, S. W., Ewen, M. E. and Sherr, C. J. (1993). Direct binding of cyclin D to retinoblastoma gene product (pRb) and pRb phosphorylation by the cyclin D-dependent kinase CDK4. *Genes Dev.* **7**, 331-342.

Knoblich, J. A., Sauer, K., Jones, L., Richardson, H., Saint, R. and Lehner, C. F. (1994). Cyclin E controls progression through S phase and its down regulation during Drosophila embryogenesis is required for the arrest of cell proliferation. *Cell* **77**, 107-120.

Koff, A., Cross, F., Fisher, A., Schumacher, J., Leguellec, K., Philippe, M. and Roberts, J. M. (1991). Human cyclin E, a new cyclin that interacts with two members of the cdc2 gene family. *Cell* **66**, 1217-1228.

Koff, A., Giordano, A., Desai, D., Yamashita, K., Harper, J. W., Elledge, S., Nishimoto, T., Morgan, D. O., Franza, R. B. and Roberts, J. M. (1992). Formation and activation of a cyclin E-Cdk2 complex during the G_1 phase of the human cell cycle. *Science* **257**, 1689-1694.

Koff, A., Ohtsuki, M., Polyak, K., Roberts, J. M. and Massague, J. (1993). Negative regulation of G_1 in mammalian cells: Inhibition of cyclin E-dependent kinase by TGF-β. *Science* **260**, 536-539.

Lees, J. A., Buchkovich, K. J., Marshak, D. R., Anderson, C. W. and Harlow, E. (1991). The retinoblastoma protein is phosphorylated on multiple sites by human cdc2. *EMBO J.* **10**, 4279-4290.

Lees, E., Faha, B., Dulic, V., Reed, S. I. and Harlow, E. (1992). Cyclin E/cdk2 and cyclin A/cdk2 kinases associate with p107 and E2F in a termporally distinct manner. *Genes Dev.* **6**, 1874-1885.

Lees, J. A., Saito, M., Vidal, M., Valentine, M., Look, T., Harlow, E., Dyson, N. and Helin, K. (1993). The retinoblastoma protein binds to a family of E2F transcription factors. *Mol. Cell Biol.* **13**, 7813-7825.

Lew, D. J., Dulic, V. and Reed, S. I. (1991). Isolation of three novel human cyclins by rescue of G_1 cyclin (Cln) function in yeast. *Cell* **66**, 1197-1206.

Lew, D. J. and Reed, S. I. (1992). A proliferation of cyclins. *Trends Cell Biol.* **2**, 77-81.

Lin, B. T.-Y., Gruenwald, S. Morla, A. O., Lee, W.-H. and Wang, J. Y. J. (1991). Retinoblastoma cancer suppressor gene product is a substrate of the cell cycle regulator of cdc2 kinase. *EMBO J.* **10**, 857-864.

Little, J. B. and Nagasawa, H. (1985). Effect of confluent holding on potentially lethal damage repair, cell cycle progression, and chromosomal aberrations in human normal and ataxia-telangiectasia fibroblasts. *Radiat. Res.* **101**, 81-93.

Ludlow, J. W., DeCaprio, J. A., Huang, C.-M., Lee, W.-H., Paucha, E. and Livingston, D. M. (1989). SV40 large T antigen binds preferentially to an underphosphorylated member of the retinoblastoma susceptibility gene product family. *Cell* **56**, 57-65.

Matsushime, H., Roussel, M. F., Ashmun, R. A. and Sherr, C. J. (1991). Colony-stimulating factor 1 regulates novel cyclins during the G_1 phase of the cell cycle. *Cell* **65**, 701-713.

Matsushime, H., Ewen, M. E., Strom, D. K., Kato, J.-Y., Hanks, S. K., Roussel, M. F. and Sherr, C. J. (1992). Identification and properties of an atypical catalytic subunit ($p34^{PSKJ3}$/cdk4) for mammalian D type G_1 cyclins. *Cell* **71**, 323-334.

Meyerson, M, Enders, G. H., Wu, C., Su, L., Gorka, C., Nelson, C., Harlow, E. and Tsai, L. (1992). A family of human cdc2-related kinases. *EMBO J.* **11**, 2909-2917.

Mihara, K., Cao, X. R., Yen, A., Chandler, S., Driscoll, B. Murphree, A. L. T'ang, A. and Fung, Y. K. (1989). Cell cycle-dependent regulation of phosphorylation of the human retinoblastoma gene product. *Science* **246**, 1300-1303.

Motokura, T., Bloom, T., Kim, H. G., Juppner, H., Ruderman, J. V., Kronenberg, H. M. and Arnold, A. (1991). A novel cyclin encoded by a Bcl1-linked candidate oncogene. *Nature* **350**, 512-515.

Nevins, J. R. (1992). E2F: A link between the Rb tumor suppressor protein and viral oncoproteins. *Science* **258**, 424-429.

Ohtsubo, M. and Roberts, J. M. (1993). Cyclin-dependent regulation of G_1 in mammalian cells. *Science* **259**, 1908-1912.

Pagano, M., Pepperkok, R., Verde, F., Ansorge, W. and Draetta, G. (1992).

Cyclin A is required at two points in the human cell cycle. *EMBO J.* **11**, 961-971.

Pines, J. and Hunter, T. (1990). Human cyclin A is adenovirus E1A-associated p60 and behaves differently from cyclin B. *Nature* **346**, 760-763.

Pines, J. and Hunter, T. (1991). Cyclin-dependent kinases: a new cell cycle motif? *Trends Cell Biol.* **1**, 117-121.

Polyak, K., Kato, J.-Y., Solomon, M. J., Sherr, C. J., Massague, J., Roberts, J. M. and Koff, A. (1994). $p27^{Kip1}$, a cyclin-Cdk inhibitor, links transforming growth factor-β and contact inhibition to cell cycle arrest. *Genes Dev.* **8**, 9-22.

Quelle, D. E., Ashmun, R. A., Shurtleff, S. A., Kato, J.-Y., Bar-Sagi, D., Roussel, M. F. and Sherr, C. J. (1993). Overexpression of mouse D-type cyclins accelerates G_1 phase in rodent fibroblasts. *Genes Dev.* **7**, 1559-1571.

Reed, S. I. (1992). The role of p34 kinases in the G_1 to S phase transition. *Annu. Rev. Cell Biol.* **8**, 529-561.

Resnitzky, D., Gossen, M., Bujard, H. and Reed, S. I. (1994). Acceleration of the G_1/S phase transition by expression of cyclins D1 and E using an inducible system. *Mol. Cell. Biol.* **14**, 1669-1679.

Sherr, C. J. (1993). Mammalian G_1 cyclins. *Cell* **73**, 1059-1065.

Slingerland, J. M., Hengst, L., Pan, C.-H., Alexander, D., Stampfer, M. R. and Reed, S. I. (1994). A novel inhibitor of cylicn/Cdk activity detected in TGF-β arrested epithelial cells. *Mol. Cell. Biol.* **14**, 3683-3694.

Withers, D. A., Harvey, R. C., Faust, J. B., Melnyk, O., Carey, K. and Meeker, T. C. (1991). Characterization of a candidate *bcl1-1* gene. *Mol. Cell. Biol.* **11**, 4846-4853.

Won, K.-A., Xiong, Y., Beach, D. and Gilman, M. Z. (1992). Growth-regulated expression of D-type cyclin genes in human diploid fibroblasts. *Proc. Nat. Acad. Sci. USA* **89**, 9910-9914.

Xiong, Y. and Beach, D. (1991). Population explosion in the cyclin family. *Curr. Biol.* **1**, 362-364.

Xiong, Y., Connolly, T., Futcher, B. and Beach, D. (1991). Human D-type cyclin. *Cell* **65**, 691-699.

Xiong, Y., Hannon, G. J., Zhang, H., Casso, D., Kobayashi R. and Beach, D. (1993). p21 is a universal inhibitor of cyclin kinases. *Nature* **366**, 701-704.

Zhu, L., van de Heuvel, S., Helin, K., Fattaey, A., Ewen, M. E., Livingston, D., Dyson, N. and Harlow E. (1993). Inhibition of cell proliferation by p107, a relative of the retinoblastoma protein. *Genes Dev.* **7**, 1111-1125.

Zindy, F., Lamas, E., Chenivesse, X., Sobczak, J., Wang, J., Fesquet, D. Henglein, B. and Brechot, C. (1992). Cyclin s is required in phase in normal epithelial cells. *Biochem. Biophys. Res. Commun.* **182**, 1144-1154.