Cell Cycle Controls: Potential Targets for Chemical Carcinogens?

by Cynthia A. Afshari\textsuperscript{1,2,3} and J. Carl Barrett\textsuperscript{1}

The progression of the cell cycle is controlled by the action of both positive and negative growth regulators. The key players in this activity include a family of cyclins and cyclin-dependent kinases, which are themselves regulated by other kinases and phosphatases. Maintenance of balanced cell cycle controls may be directly linked to genomic stability. Loss of the checkpoints involved in cell cycle control may result in unrepaired DNA damage during DNA synthesis or mitosis leading to genetic mutations and contributing to carcinogenesis.

This overview provides a general review of cell cycle control and describes how chemicals may interfere with these controls, leading to neoplastic development. For us to discuss mechanisms of cell cycle control, the cell cycle must first be defined. The most basic cell cycle, which exists in the cleavage stage of frog embryos, consists of only two phases, DNA synthesis (S phase) and mitosis (M phase) or cell division (1). This cell cycle lacks two additional phases or gaps (G\textsubscript{1} and G\textsubscript{2}) observed in more complex cycles in adult cells and most embryonic cells. The G\textsubscript{1} and G\textsubscript{2} gaps, intervening between the M- and S-phase, allow growth control points to regulate cell size and cell number and to monitor the cell’s environment for nutrients and growth signals (2,3). The existence of G\textsubscript{1} and G\textsubscript{2} phases also allows the cell to ensure that certain intracellular events are completed before the cell progresses to the next phase of the cell cycle (4). For example, DNA replication and chromosome segregation must be completed before a cell continues through the cell cycle. These controls have been referred to as cell cycle checkpoints (4). Several G\textsubscript{1} checkpoints have been proposed for mammalian fibroblasts (5). The most studied of these is the R, or restriction, point (6). Cells that do not have sufficient nutrients may arrest in late G\textsubscript{1} at this point. It has been proposed that critical proteins must accumulate to a certain level before a cell can pass the R point to enter S-phase and that some cancer cells may stabilize these proteins and therefore override these checkpoints. This may lead to infidelity in replication and provide a partial explanation for the observance of the high level of chromosome aberrations in cancer cells (4).

Cell Cycle Control Proteins

Several classes of proteins are important in cell cycle control. Progression through the cell cycle depends on the action of a family of kinases known as cyclin-dependent kinases (cdk) and the interaction of these kinases with another class of proteins called cyclins. The activity of these complexes in turn appears to be regulated by various phosphatases and kinases.

The first member of the cdk family identified was the S. pombe cdc2 gene (Table 1). Cell division cycle (cdc) mutants in yeast have mutations in specific genes involved in cell cycle progression. Conditional mutations of these genes results in cell arrest at very specific points of the cell cycle when the mutants are placed under restrictive conditions (7). Study of these mutants led to the cloning of the cdc2 gene, which codes for a protein (8) that is a key regulator of the cell cycle in eukaryotic cells including yeast and human cells. In fact, the human cdc2 gene was cloned by functional complementation of the yeast cdc2 mutant by human cDNA (9), indicating that this gene is highly conserved between yeast and humans. In addition, the cdc2 gene has been cloned in mouse (10) and several other species (11-13). The conservation of this gene suggests that it plays an important, fundamental role in growth and division. The cdc2 protein (p34\textsuperscript{cdc2}) is a serine-

\textsuperscript{1}National Institute of Environmental Health Sciences, P.O. Box 12233, Research Triangle Park, NC 27709.
\textsuperscript{2}Curriculum of Toxicology, University of North Carolina, Chapel Hill, NC.
\textsuperscript{3}Present address: Center for the Study of Aging and Human Development, Duke University Medical School, P.O. Box 3003, Durham, NC 27705.

Address reprint requests to J.C. Barrett, National Institute Environmental Health Sciences, P.O. Box 12233, Research Triangle Park, NC 27709.

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The kinase activation of p34<sup>cdc2</sup> is subject to negative control by phosphorylation on tyrosine 15 and dephosphorylation of this site is required for activation (33). The phosphorylation state of p34<sup>cdc2</sup> fluctuates through the cell cycle (34). Two yeast gene products, the wee1 kinase (35–37) and the cdc25 phosphatase (38,39), are responsible in part for this regulation. 

cdc25 is believed to be the factor responsible for initiation of mitosis, which is dependent upon completion of DNA replication. p80<sup>cdc25</sup> is the tyrosine phosphatase that activates p34<sup>cdc2</sup> by dephosphorylation of the tyrosine 15 residue of p34<sup>cdc2</sup> when it is complexed to cyclin B (39,40–42). Several cdc25 genes have been identified, suggesting that a family of these proteins exists and association between cyclin B and cdc25 has been observed (43,44). Therefore, one function of cyclin B may be to target p80<sup>cdc25</sup> to p34<sup>cdc2</sup> for G/M activation. The wee1+ gene product negatively regulates entry into mitosis (36).

The p107<sup>weel+</sup> protein is a dual function kinase that phosphorylates serine, threonine, as well as tyrosine residues (45). Wee1+ and a related gene product, mik1, are responsible for phosphorylating p34<sup>cdc2</sup> on tyrosine 15, thereby inactivating it (35,37). Analysis of yeast wee1 mutants that have lost cdc25 control have lost mitotic dependency on completion of DNA replication (46). The gene was first cloned in fission yeast and a human wee1-like gene has been cloned by complementation of human cDNA into a yeast mutant (47).

In addition to p107<sup>weel</sup> and p80<sup>cdc25</sup>, activated p34<sup>cdc2</sup> binds a protein of unknown function, p13<sup>sucl</sup> (48). It is known, however, that binding of p34<sup>cdc2</sup> to p13<sup>sucl</sup> is required for p34<sup>cdc2</sup> activity (49,50). It has been proposed that p13<sup>sucl</sup> may act as a facilitator of the formation or localization of the p34<sup>cdc2</sup> kinase complex (49).

The expression of the wee1+, sucl, cyclins A and B as well as cdc25 homologs in human cells suggest that not only is the structure of cdc2 conserved across species, but also that its regulation is conserved, further indicating that p34<sup>cdc2</sup> plays a basic and important role in growth control. Very little is known about the in vivo functions of the cdc2/cyclin complexes; however, it has been shown that this kinase is involved in the breakdown of the nuclear envelope during mitosis (51,52).

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**Table 1. Characteristics of p34<sup>cdc2</sup>**

| p34<sup>cdc2</sup> is required for G1/S and G2/M transitions in the cell cycle. | Protein levels of p34<sup>cdc2</sup> are usually constant during the cell cycle but activity is periodic. |
|---|---|
| cdc2 protein is the 34 Kd catalytic subunit of a serine-threonine protein kinase complex. | Kinase activity is regulated by protein–protein interactions, particularly with different members of the cyclin family, and also by phosphorylation and dephosphorylation. |
| Kinase phosphorylates a number of substrates that are possibly involved in regulation of specific events in the cell cycle. | This protein kinase complex is responsible for M-phase-specific histone H-1 kinase activity. |
| Homologous to important cell control gene, CDC25, of the budding yeast Saccharomyces cerevisiae, which was isolated as a mutation that arrested cells at "start" in G<sub>1</sub>. | Start defines a central control point in yeast at which the cell decides to continue to grow and divide, to enter into stationary phase, or to mate. This is the first point in the cell cycle under genetic control in S. cerevisiae. |
| cdc2 is a member of a family of genes that are cyclin-dependent kinases (cdk). | CDC25<sup>weel</sup> was cloned by complementation of cdc2 ts mutant in fission yeast (S. pombe) with human cDNA. |

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**Table 2. Characteristics of cyclin proteins**

| Identified in marine invertebrates as two proteins (cyclins A and B) whose abundance oscillates in early invertebrate embryonic cell cycles and regulate G1/M transition. |
|---|---|
| A family of cyclins exists that regulates progression through the cell cycle. |
| Cyclin A is required for two points in the cell cycle, S-phase and G2/M phase. |
| Different cyclins (G1 cyclins) regulate the G1/S transition in yeast (CLN1-3), and at least 5 proteins (cyclins C, D1-3, and E) are identified as candidate G1 cyclins in mammalian cells. |
| There are also other cyclins involved in mitosis in yeast (e.g., MCS). |
| Cyclins combine with p34<sup>cdc2</sup> and other cdks to form an active cdc2 kinase. |
| Cyclins are involved in regulation of phosphorylation/dephosphorylation of p34<sup>cdc2</sup>. |
| Cyclins are degraded rapidly at specific times in the cell cycle by proteolysis mediated by the ubiquitin pathway. |
| Cyclins are altered in certain cancer cells. |
Studies of cell-free extracts show that p34\textsuperscript{cdk2} may be involved in complex formation at the replication origin prior to initiation of DNA synthesis (53). Several proteins have been identified as substrates for the p34\textsuperscript{cdk2} kinase. These include the retinoblastoma protein (54,56), nucleolar proteins (57), c-src (58), histone H1 (59,60) and other proteins (61).

Other cyclin-dependent kinases have been described. Human cdk2 was discovered as a target for binding by the E1A protein of a DNA tumor virus. The p33\textsuperscript{cdk2} protein, like p34\textsuperscript{cdk2}, has protein kinase activity and binds cyclin A (62). It also binds G1 cyclins, cyclin E (63) and possibly cyclin D (28). Its kinase activity peaks in late G1 or early S phase indicating that it plays an important role at a point earlier in the cell cycle than p34\textsuperscript{cdk2} (64). In addition, cdk2 is part of a complex formed with the transcription factor, E2F, indicating its kinase activity may be important in gene regulation (65,66). Pines and Hunter have proposed that the functions of cdks are critical for the eukaryotic cell cycle and are required to traverse checkpoints (28).

Cyclin A association with p33\textsuperscript{cdk2} has been shown to be required for entry into DNA synthesis in mammalian cells (67,68). In addition, several new cyclins that appear to play a role in G1 have been cloned. Human cyclin D1 was cloned for its ability to complement a yeast deficient in a G1 cyclin function and also as a gene induced late in G1 in growth factor (CSF-1) stimulated mouse macrophages (69,70). This gene is the same as the \textit{PRADI} oncogene that is overexpressed in parathyroid tumors (71). Cyclins C and E are two other cyclin molecules expressed during G1 (72,73). Cyclin E protein is associated with a histone kinase activity that is most likely derived from its interaction with p33\textsuperscript{cdk2} (74). Although the exact functions of these different G1 cyclin/kinase complexes are unknown, the nature of their cycle-dependent expression indicates their importance in the G1/S transition.

**Cell Cycle Checkpoints and Perturbations**

While the functions of these cell cycle control proteins are just beginning to be understood, perturbations of these controls are already being observed during abnormal growth states such as transformation. For example, cyclin A and cdk2 are both targets for binding by DNA tumor viral protein E1A (62). In addition, the hepatitis B virus is integrated into the cyclin A gene in a hepatocellular carcinoma (75). As previously mentioned, cyclin D1 is overexpressed in parathyroid tumors (71). Several cyclins have been shown to be overexpressed in breast cancer (76).

However, these control proteins are not the only potential targets for carcinogens or tumor promoters. In addition to cell cycle control proteins involved in normal cell cycle progression, there are other proteins that are important in the regulation of cell cycle checkpoints in response to agents that damage DNA or perturb the cell cycle. For example, the \textit{RAD 9} gene of \textit{S. cerevisiae} is responsible for arresting cells after DNA damage by X-irradiation. The \textit{RAD 9} gene is not required for cell growth, but \textit{RAD 9} mutants fail to arrest after treatment with radiation and therefore have no time to repair DNA damage (4). In addition to rad 9, the wee1 kinase has been shown to be required for mitotic delay after irradiation (77). Therefore, mutation of this gene not only perturbs normal cell cycle progression but also makes a cell more susceptible to radiation-induced damage. Recently, it has been shown in human cells that p53 protein levels increase in response to radiation damage (78,79). This leads to the hypothesis that p53 may be acting similarly to rad 9 as a checkpoint in order to inhibit cell division until repair has occurred. However, p53 is involved in a G1 checkpoint whereas \textit{RAD 9} is a G2 checkpoint. Loss of this checkpoint may lead to an increase in genetic instability (80).

In addition to aberrations in repair after exogenous damage, loss of cell cycle checkpoints can increase the rate of “spontaneous” mutations. For example, \textit{RAD 9} mutants in yeast have a 21-fold elevated rate of chromosome loss (81) and mutant p53 human and mouse cells have a several hundredfold elevation in the rate of gene amplification (80,82). Increased genetic instability may also result from chemical treatments that block the action of proteins involved in checkpoints. For example, caffeine blocks upregulation of p53 protein in irradiated cells and prevents radiation-induced G1 growth arrest (83). Okadaic acid, a tumor promoter, inhibits phosphatases that regulate G2/M checkpoints and can induce mitotic abnormalities (84–85). Chemical carcinogens may also mutate checkpoint genes, and loss of these protein functions might predispose a cell to successive mutational events. This is consistent with a model in which the occurrence of one mutation in a cell increases susceptibility for a second mutation. Neoplastic development is a multistep process requiring at least four to five distinct steps (86). Since the probability that a cell will acquire multiple defects is low, the existence of predisposing mutations may explain the ontogeny of many adult cancers.

**Role of Cell Proliferation in Carcinogenesis**

Cell proliferation can influence carcinogenesis by various mechanisms (Table 3). This has led to the hypothesis that cell proliferation itself may be carcinogenic and carcinogens that increase cell proliferation may be operating exclusively by this mechanism. The failure to detect a measurable mutagenic activity associated with nongenotoxic carcinogens indicates that these chemicals may act by alternative mechanisms of action, increasing cell proliferation being one possibility. This hypothesis is supported by the fact that in some species many types of cancers may arise spontaneously. Normal cell division results in a low level of
spontaneous errors during DNA replication, and spontaneous DNA damage can result from cytosine deamination at physiological temperatures, from oxidative damage associated with normal cellular physiology, and from mutagens in food, air, or water (87). Thus, mutations occur "spontaneously" from normal cellular processes. There are risk factors for human cancers (e.g., hormones) that also influence the rate of cell proliferation in target tissue (88). However, mechanisms in addition to cell proliferation should be considered for these risk factors.

Before cell proliferation can be accepted as the causative mechanism for certain carcinogens, several facts should be considered. First, many toxic and/or hyperplastic stimuli are not carcinogenic (89–91). Second, cell division occurs frequently in all organisms; therefore, it is not clear whether cell division is limiting in the carcinogenic process. This, of course, depends on the target tissue. Furthermore, cell division of initiated or intermediate cells may occur at quite different rates than division of normal cells. Finally, the observation that multiple mutations are involved in the development of many neoplasms may suggest that even a weak mutagenic response, which is below the level of detection of current assays, is sufficient to influence the neoplastic process in a specific target tissue. This is a plausible explanation for certain nongenotoxic carcinogens, some of which may act by indirect mutagenic processes.

**Conclusion**

The cell cycle is controlled by a network of proteins whose activity is intricately regulated. DNA synthesis and cell division are tightly coupled to these controls. The observations presented here support the hypothesis that growth arrest points exist to control genetic fidelity and stability. Disruption of growth arrest checkpoints by mutation or by chemical treatment may lead to increased cell growth and genetic instability (Table 3). Finally, chemicals that induce cell proliferation and genetic instability by interfering in regulatory checkpoints, thus disturbing the cell's process of "checks and balances," are more likely to cause cancer than chemicals that are only mitogenic.

**REFERENCES**

1. Kirschner, M. Newport, J., and Gerhart, J. The timing of early development events in Xenopus. Trends Genet. 1: 41–47 (1985).
2. Enoch, T., and Nurse, P., Coupling M phase and S phase: controls maintaining the dependence of mitosis on chromosome replication. Cell 65: 921–923 (1991).
3. Cross, F., Roberts, J., Weintrob, H. Simple and complex cell cycles. Annu. Rev. Cell Biol. 5: 341–395 (1989).
4. Hartwell, L. H., Weinert, T. A. Checkpoints: controls that ensure the order of cell cycle events. Science 246: 629–634 (1989).
5. Pardee, A. B. G events and regulation of cell proliferation. Science 246: 603–607 (1989).
6. Pardee, A. B. A restriction point for control of normal animal cell proliferation. Proc. Natl. Acad. Sci. USA 71: 1266–1290 (1974).
7. Hartwell, L. H. Saccharomyces cerevisiae cell cycle. Bacteriol. Rev. 38: 164–198 (1974).
8. Beach, D., Durkacz, B., Nurse P. Functionality homologous cell cycle control genes in fission yeast and budding yeast. Nature 300: 706–709 (1982).
9. Lee, M. G., Nurse P. Complementation used to clone a human homologue of the fission yeast cell cycle control gene cdc2. Nature 327: 31–35 (1987).
10. Spurr, N. K., Gough, A. C., Lee M. G. Cloning of the mouse homologue of the yeast cell cycle control gene cdc2. J. DNA Seq. Mapp. 1: 49–54 (1990).
11. Hirt, H., Pay, A., Gyorgyevi, J., Bako, L., Nemeth, K., Bogre, L., Schwyepy, R. J., Heberle-Bors, E., Dudit, D. Complementation of a yeast cell cycle mutant by an alfalfa cDNA encoding a protein kinase homologous to p34cdc2. Proc. Natl. Acad. Sci. USA 88: 1636–1640 (1991).
12. Colasanti, J., Tyers, M., Sundraesan, V. Isolation and characterization of cDNA clones encoding a functional p34cdc2 homologue from Zea mays. Proc. Natl. Acad. Sci. USA 88: 3377–3381 (1991).
13. Feiler, H. S., Jacob, T. W. Cell division in higher plants: a cdc2 gene, its 34 kDa product, and histone H1 kinase activity in pea. Proc. Natl. Acad. Sci. USA 87: 5397–5401 (1990).
14. Lee, M. G., Norbury, C. J., Spurr, N. K., Nurse, P. Regulated expression and phosphorylation of a possible mammalian cell cycle control protein. Nature 333: 676–679 (1988).
15. Richter, K. H., Afshari, C. A., Annab, L. A., Burkhardt, B. A., Owen, R. D., Boyd, J., Barrett, J. C. Down-regulation of cdc2 in senescent human and hamster cells. Cancer Res. 51: 6010–6013 (1991).
16. Stein, G. H., Drullinger, L. F., Robetorye, R. S., Pereira-Smith, O. M., Smith, J. R. Senescent cells fail to express cdc2, cycA, and cycB in response to mitogen stimulation. Proc. Natl. Acad. Sci. USA 88: 11012–11016 (1991).
17. Hayes, T. E., Valtz, N. L. M., McKay, R. D. G. Down regulation of cdc2 upon terminal differentiation of neurons. New Biol. 3: 253–269 (1991).
18. Akhurst, R. J., Flavin, N. B., Worden, J., Lee, M. G. Intracellular localisation and expression of mammalian CDC2 protein during myogenic differentiation. Differentiation 40: 36–41 (1989).
19. Riabowol, K., Draetta, G., Brizuela, L., Vandre, D., Beach, D. The cdc2 kinase is a nuclear protein that is essential for mitosis in mammalian cells. Cell 57: 393–401 (1989).
20. Broek, D., Bartlett, R., Crawford, K., Nurse, P. Involvement of p34cdc2 in establishing the dependency of S phase on mitosis. Nature 349: 388–393 (1991).
21. Fang, F., Newport, J. W. Evidence that the G1-S and G2-M transitions are controlled by different cdc2 proteins in higher eukaryotes. Cell 66: 731–742 (1991).
22. Nurse, P. Universal control mechanism regulating onset of M phase. Nature 344: 503–506 (1990).
23. Moris, A. O., Draetta, G., Beach, D., Wang, J. Y. J. Reversible tyrosine phosphorylation of cdc2: dephosphorylation accompanies activation during entry into mitosis. Cell 58: 193–203 (1989).
24. Draetta, G., Frieda-Worms, H., Morrison, D., Druker, B., Roberts, T., Beach, D. Human cdc2 protein kinase is a major

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**Table 3. Mechanisms by which chemicals affecting cell proliferation might influence carcinogenesis.**

| Mechanism                                                                 | Influence on Carcinogenesis |
|--------------------------------------------------------------------------|----------------------------|
| Increase fixation and expression of premutagenic DNA lesions.             | Decrease susceptibility     |
| Increase the number of initiated cells occurring spontaneously during cell division. | Increase the number of initiated cells |
cell-cycle regulated tyrosine kinase substrate. Nature 336: 738–744 (1988).

25. Draetta, G., Beach, D. Activation of cdk2 protein kinase during mitosis in human cells: cell-cycle-dependent phosphorylation and subunit rearrangement. Cell 54: 17–26 (1988).

26. Dalton, S. Cell cycle regulation of the human cdc2 gene. EMBO J. 11: 1797–1804 (1992).

27. Welch, P. J., Wang J. Y. J. Coordinated synthesis and degradation of cdk2 in the mammalian cell cycle. Proc. Natl. Acad. Sci. USA 89: 3083–3087 (1992).

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

29. Glotzer, M., Murray, A. W., Kirschner M. Cyclin is degraded by the ubiquitin pathway. Nature 349: 132–138 (1990).

30. Murray, A. W., Kirschner, M. W. Dominos and clocks: the union of two views of the cell cycle. Science 246: 614–621 (1990).

31. Meijer, L., Azzi, L., Wang, J. Y. J. Cyclin B targets p34^cdk2 for tyrosine phosphorylation. EMBO J. 10: 1545–1554 (1991).

32. Pines, J., Hunter, T. Human cyclin A is an adenosine E1 A-associated protein p60 and behaves differently from cyclin B. Nature 346: 760–763 (1990).

33. Gould, K. L., Nurse, P. Tyrosine phosphorylation of the fission yeast cdc2 protein kinase regulates entry into mitosis. Nature 349: 808–811 (1991).

34. Krek, W., Nigg, E. A. Differential phosphorylation of vertebrate p34^cdk2 kinase at the G1/S and G2/M transitions of the cell cycle: identification of major phosphorylation sites. EMBO J 10: 305–316 (1991).

35. Lundgren, K., Waiworth, N., Boorer, R., Dambski, M., Kirshner, M., Belach, D. mik1 and wee1 cooperate in the inhibitory tyrosine phosphorylation of cdc2. Cell 64: 1111–1122 (1991).

36. Featherstone, C., Russell, P. Fission yeast p107^es is mitotic inhibitor is a tyrosine/serine kinase. Nature 349: 808–811 (1991).

37. Parker, L. L., Atherton-Fessler, S., Lee, M. S., Ogg, S., Falk, J. L., Swenson, K. I., Piwinka-Worms, H. Cyclin promotes the tyrosine phosphorylation of p34^cdk2 in a cdc15-dependent manner. EMBO J. 10: 1235–1239 (1991).

38. Millar, J. B. A., Russell, P. The cdc25 M-phase inducer: an unconventional protein phosphatase. Cell 68: 407–410 (1992).

39. Gautier, J., Solomon, M. J., Boorer, R. N., Bazan, J. F., Krishner, M. W. cdc25 is a specific tyrosine phosphatase that directly activates p34^cdk2. Cell 67: 197–211 (1991).

40. Millar, J. B. A., McGowan, C. H., Lenoar, G., Jones, R., Russell, P. p34^cdk2 mitotic inducer is the tyrosine phosphatase that activates p34^cdk2 kinase in fission yeast. EMBO J. 10: 4301–4309 (1991).

41. Strausfeld, U., Labbe, J. C., Fesquet, D., Cavadores, J. C., Picard, A., Sadhu, K., Russell, P., Doree, M. Dephosphorylation and activation of a p34^cdk2/cyclin B complex in vitro by human cdc25 protein. Nature 351: 242–245 (1991).

42. Gould, K., Moreno, S., Tonks, N. K., Nurse, P. Complementation of the mitotic activator, p80^cdk1, by a human protein-tyrosine phosphatase. Science 250: 1573–1575 (1990).

43. Jessus, C., Beach, D. Oscillation of MPF is accompanied by periodic association between cdc25 and cdc2/cyclin B. Cell 68: 323–332 (1992).

44. Galaktionov, K., Beach, D. Specific activation of cdc25 tyrosine phosphatase by B-type cyclins: evidence for a role of tyrosine phosphorylation. Cell 67: 1181–1194 (1991a).

45. Parker, L. L., Atherton-Fessler, S., Piwinka-Worms, H. p107^es is a dual-specificity kinase that phosphorylates p34^cdk2 on tyrosine 15. Proc. Natl. Acad. Sci. USA 88: 2917–2921 (1991).

46. Enoch, T., Nurse, P. Mutation of fission yeast cell cycle control genes abolishes dependence of mitosis on DNA replication. Cell 66: 665–673 (1991).

47. Igarashi, M., Nagata, A., Jinno, S., Suto, K., Okayama, H., Wee1-like gene in human cells. Nature 353: 80–83 (1991).

48. Draetta, G., Brizuela, L., Potashkin, J., Beach, D. Identification of p34 and p18/cdc1 in fission yeast cell division cycle as a component of the p34^cdk2 protein kinase. EMBO J. 6: 3507–3514 (1987).

49. Draetta, G., Bloom, T., Kim, H. B., Japper, R., Ruderman, J. V., Kronenberg, H. M., Arnold, A. A novel cyclin encoded by a bcl-1-linked candidate oncogene. Nature 350: 512–515 (1991).
72. Lew, D. J., Dxic, V., Reed S. I. Isolation of three novel human cyclins by rescue of G1 cyclin function in yeast. Cell 66: 1197–1206 (1991).
73. Leopold, P., O’Farrell, P. H. An evolutionarily conserved cyclin homolog from Drosophila rescues yeast deficient in G1 cyclins. Cell 66: 1207–1216 (1991).
74. Koff, A., Cross, F., Fisher, A. Shumacher, J., Leguellec, K., Philippe, M., Roberts, J. M. Human cyclin E, a new cyclin that interacts with two members of the CDC2 gene family. Cell 66: 1217–1228 (1991).
75. Wang, J., Chenivesse, X., Henglein, B., Brechet, C. Hepatitis B virus integration in a cyclin A gene in a hepatocellular carcinoma. Nature 343: 555–557 (1990).
76. Keyomarsi, J., Pardee, A. B. Redundant cyclin overexpression and gene amplification in breast cancer cells. Proc. Natl. Acad. Sci. U.S.A. 90: 1112–1116 (1993).
77. Rowley, R., Hudson, J., Young, P.G. The wee1 protein kinase is required for radiation-induced mitotic delay. Nature 356: 353–355 (1992).
78. Kuerbitz, S. J., Plunkett, B. S., Walsh, W. V., Kastan, M. B. Wild-type p53 is a cell cycle checkpoint determinant following irradiation. Proc. Natl. Acad. Sci. U.S.A. 89: 7491–7495 (1992).
79. Kastan, M. B., Zhan, Q., El-Deiry, W. S., Carrier, F., Jacks, T., Walsh, W. V., Plunkett, B. S., Vogelstein, B., Fornace, A. J. A mammalian cell cycle checkpoint pathway utilizing p53 and GADD45 is defective in ataxia-telangiectasia. Cell 71: 587–597 (1992).
80. Livingstone, L. R., White, A., Sprouse, J., Livanes, E., Jacks, T., Tisty, T. D. Altered cell cycle arrest and gene amplification potential accompany loss of wild-type p53. Cell 70: 323–336 (1992).
81. Weinert, T. A., Hartwell, L. H. Characterization of RAD9 of Saccharomyces cerevisiae and evidence that its function acts posttranslationally in cell cycle arrest after DNA damage. Mol. Cell. Biol. 10: 6554–6564 (1990).
82. Yin, Y., Tainsky, M. A., Bischoff, F. Z., Strong, L. C., Wahl, G. M. Wild-type p53 restores cell cycle control and inhibits gene amplification in cells with mutant p53 alleles. Cell 70: 967–968 (1992).
83. Kastan, M. B., Onyekwere, O., Sidransky, D., Vogelstein, B., Craig, R. W. Participation of p53 protein in the cellular response to DNA damage. Cancer Res. 51: 6304–6311 (1991).
84. Suganuma, M., Fujiki, H., Suguri, H., Yoshizawa, S., Hirota, M., Nakayasu, M., Ojika, M., Wakamatsu, K., Yamada, K., Sugimura, T. Okadaic acid: an additional nonphorbol-12-tetradecanoate-13-acetate-type tumor promoter. Proc. Natl. Acad. Sci. U.S.A. 85: 1768–1771 (1988).
85. Zheng, B., Woo, C. F., Kuo, J. F. Mitotic arrest and enhanced nuclear protein phosphorylation in human leukemia K562 cells of okadaic acid, a potent protein phosphatase inhibitor and tumor promoter. J. Biol. Chem. 266: 10031–10034 (1991).
86. Vogelstein, B., Fearon, E. R., Hamilton, S., Kern, S., Preisinger, A., Leppert, M., Nakamura, Y., White, R., Smiths, A., Bos, J. Genetic alterations during colorectal-tumor development. New Engl. J. Med. 319: 525–532 (1988).
87. Loeb, L. A. Endogenous carcinogenesis: molecular oncology into the twenty-first century—presidential address. Cancer Res. 49: 5489–5496 (1989).
88. Preston-Martin, S., Pike, M. C. Ross, R. K., Jones, P. A., Henderson, B. E., Increased cell division as a cause of human cancer. Cancer Res. 50: 7415–7421 (1990).
89. Ledda-Columbano, G. M., Columbano, A., Curto, M., Coni, M. G. E., Sarma, D. S. R., Pani, F. Further evidence that mitogen-induced cell proliferation does not support the formation of enzyme-altered islands in rat liver by carcinogens. Carcinogenesis 10: 847–850 (1989).
90. Hoel, D. G., Haseman, J. K., Hogan, M. D., Huff, J., McConnell, E. E. The impact of toxicity on carcinogenicity studies: implications for risk assessment. Carcinogenesis 9: 2045–2052 (1988).
91. Huff, J. Chemical toxicity and chemical carcinogenesis. Is there a causal connection? In: Mechanisms of Carcinogenesis in Risk Evaluations H. Vainio, P. Magee, D. McGregor, A. J. McMichael, Eds, (IARC Scientific Publications No. 116) International Agency for Research on Cancer, Lyon, 1992, in press.