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Apoptosis and the Cell Cycle

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Abstract In this review, we consider apoptosis as a process intimately linked to the cell cycle. There are several reasons for thinking of apoptosis as a cell cycle phenomenon. First, within the organism, apoptosis is almost exclusively found in proliferating tissues. Second, artificial manipulation of the cell cycle can either prevent or potentiate apoptosis, depending on the point of arrest. Data from such studies have suggested that molecules acting late in G1 are required for apoptosis. Since passage through late G1 into S phase in mammalian cells is known to be regulated by p53 and by activation of cyclin-dependent kinases, we also examine recent studies linking these molecules to the apoptotic pathway.

Key words: DNA replication, chromatin condensation, premature mitosis, cyclin A, p53, cyclin-dependent kinases

Not all cell deaths are accidental. In animal development, proper shape and function require cell death as well as cell division. For example, fingers and toes are formed from the death of cells in the interdigital tissue [Kerr et al., 1987; Novack and Korsmeyer, 1994]. The elaboration of even more complex structures also depends on cell death. Formation of the T and B cell repertoires of the immune system is dependent on negative selection (elimination of nonreactive or self-reactive cells) as much as positive selection [Cohen, 1991]. Similar forces are at work in shaping the nervous system [Raff, 1992]. In development of the nematode *Caenorhabditis elegans,* exactly 131 cells die according to a precisely regulated genetic program [Yuan and Horvitz, 1990]. Cell death is also necessary as a protective mechanism. Examples include the directed lysis of infected cells, foreign cells, or incipient neoplasms by the immune system [Bishop and Whiting, 1983; Duke, 1991; Squier and Cohen, 1994] and the autolysis of immune cells themselves following damage by γ radiation [Umansky, 1991]. It is striking that in these various cases of cell death, there exist features sufficiently unique as to constitute a well-defined cytological entity, known as apoptosis: the form of cell death in which the cell is destroyed from within [Kerr et al., 1972; Wyllie et al., 1980]. A cardinal feature of apoptotic cells is the appearance of highly condensed chromatin, segregated into sharply defined bodies within an intact nuclear envelope. Often, the DNA is digested by endonucleases, making detection of DNA fragments a commonly used assay for apoptosis [Arends et al., 1990]. The cell shrinks and condenses, fragmenting into multiple, membrane-bound bodies (apoptotic bodies) which are eventually engulfed by surrounding cells—thus removed without inflammation and attendant damage to surrounding tissue. It is this constellation of features that is referred to as apoptosis.

The thought that multicellular organisms have evolved genetic mechanisms for promoting cell death presents something of a puzzle—how might genes be selected that have death as a phenotype? One way is by linking cell death to cell proliferation. Whereas in yeast essentially all proliferation controls ultimately feed into a single gene, *cdc2* in *S. pombe* or *CDC28* in *S. cerevisiae,* in multicellular eukaryotes cell cycle control is split up amongst a dozen *cdc2/CDC28* homologs, whose products are referred to as cyclin-dependent kinases (CDKs) because of their dependence on a cyclin subunit for full activity and cell cycle regulation. If independent activation of several CDK-cyclin complexes were required for cell division to proceed, activation of more or fewer than the proper number could be fatal. Depending then on the physiological environment, withholding activating stimuli (hor-

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mones, growth factors, cytokines) or supplying inappropriate stimuli (cell contact, antigen binding, receptor ligation) might lead to incorrect timing of CDK activation. In this scenario, the "death genes" are then actually a subset of the genes whose normal function is to mediate cell proliferation, and apoptosis is an incomplete, abnormal cell division [Ucker, 1991]. Such a hypothesis suggests that it might be possible to induce apoptosis by premature activation of CDKs by chemical or other artificial means. In yeast, mutations in regulatory genes impinging on *cdc2/CDC28* can lead to premature activation of cdc2/CDC28, which brings about a "mitotic catastrophe": a premature mitosis in which chromatin condensation, spindle formation, and abortive division is disastrous for the cell. In mammalian cells, the morphological similarities between apoptosis and premature mitosis are quite suggestive of a role for CDKs in apoptosis [Rubin et al., 1993; Sen and D'Incalci, 1992; Ucker, 1991]; in fact, such a role has recently been demonstrated [Meikrantz et al., 1994; Shi et al., 1994]. In this review, we discuss apoptosis as a process intimately linked to the cell cycle.

APOPTOSIS AS A CELL CYCLE PHENOMENON

Association With Proliferation

In vivo, apoptosis is detected primarily in proliferating tissues [Kerr and Harmon, 1991; Wyllie et al., 1980]. Anywhere in the body where cells are dividing, apoptotic bodies are to be found: at low frequency in self-renewing tissues (like intestinal crypt) [Potten, 1977], in epithelium of the adrenal cortex [Wyllie et al., 1973a,b], in the differentiating spermatogonium [Allan et al., 1987], and in the germinal centers of lymph nodes [Swartzendruber and Congdon, 1963]. Apoptosis becomes particularly evident after periods of rapid proliferation, and is thus detected in mammary tissue following weaning [Marti et al., 1994; Walker et al., 1989], in the endometrium at estrus [Gerschenson and Rotello, 1991; Sandow et al., 1979; Otsuki et al., 1994], during ovarian follicular atresia [O'Shea et al., 1978], and in numerous other cases [e.g., Kerr and Harmon, 1991; Kerr et al., 1972; Ledda-Columbano and Columbano, 1991; Wyllie et al., 1980]. As might be expected, apoptosis is a prominent feature in malignancies [Moore, 1987] and occurs at increased frequency in preneoplastic tissues [Ledda-Columbano and Columbano, 1991]. In these cases, apoptosis is thought to serve a compensatory role: balancing an increase in cell number due to proliferation with cell loss due to death. The role of apoptosis in maintaining tissue homeostasis is well-illustrated in regression of liver tissue following lead nitrate- or cyproterone acetate-induced hyperplasia [Bursch et al., 1985; Columbano et al., 1985]. Administration of either mitogen leads to an increase in organ size due to massive cell proliferation. Upon withdrawal of the mitogen, the hyperplastic tissue regresses and the organ returns to normal size. Regression is entirely due to apoptotic death.

Both positive and negative signals trigger cells to stop proliferating and undergo apoptosis. Positive signals include antigen receptor ligation of immature lymphocytes [Green and Scott, 1994], thyroxine-induced regression of larval components in amphibian metamorphosis [Yoshizato, 1989], glucocorticoid-induced thymocyte death [Wyllie, 1980], and absorption of the Müllerian structures in the male fetus in response to Müllerian-inhibiting substance [Glucksmann, 1951; Saunders, 1966]. Negative signaling comprises withdrawal of necessary growth factors. For example, sympathetic neurons depend on a continuous supply of nerve growth factor from the cells they innervate [Raff, 1992; Rubin et al., 1993], while cells in the ventral prostate rapidly undergo apoptosis if testosterone is removed by castration [reviewed in Buttyan, 1991]. Survival factor dependence is also found in tumor cell lines. For example, mouse myeloma lines will undergo apoptosis following withdrawal of IL-2 and IL-6 [Colotta et al., 1992]. Interestingly, apoptosis induced by factor withdrawal is associated with induction of proliferation-associated genes [Buttyan et al., 1988; Colotta et al., 1992]. This topic is discussed in greater detail below.

Cell Cycle Arrest and Apoptosis

Ultimately, the signals described above must impinge on the cell cycle if proliferation is to be halted and cells are to die. How this is accomplished requires understanding where in the cell cycle this intervention occurs. We consider two types of analysis: 1) cell cycle effects of physiological mediators of apoptosis, and 2) effects of artificially imposed cell cycle synchrony on apoptosis induced by other agents.

1. Cell cycle phase-specific death induced by physiological mediators of apoptosis. The WEHI-231 line is a murine B cell lymphoma which retains many of the characteristics of immature B cells [Boyd and Schrader, 1981]. It displays surface IgM but not IgD, and

does not secrete immunoglobulin. When treated with anti-IgM antibodies, mimicking antigen-induced surface IgM cross-linking, these cells die by apoptosis after approximately 24–48 h [Benhamou et al., 1990; Hasbold and Klaus, 1990]. Flow cytometry revealed anti-IgM-treated cells were blocked from entering S phase: they ceased incorporating [^{3}H]thymidine into DNA [Scott et al., 1985] and did not phosphorylate pRB [Maheswaran et al., 1991; Warner et al., 1992]. Growth arrest in G1 was apparent within 12 h, well before the morphological changes characteristic of apoptosis had occurred. Antibody treatment of G1 cells, obtained by centrifugal elutriation, prevented entry into S phase with arrest very close to the G1-S border [Scott et al., 1986].

T cell hybridomas are also sensitive to activation-induced death following ligation of their antigen receptors [Green and Scott, 1994]. Following stimulation, they rapidly accumulate in G1 prior to the appearance of an apoptotic morphology [Ashwell et al., 1987]. The ability of antisense c-*myc* oligonucleotides to halt growth and prevent apoptosis in such cells [Shi et al., 1992] implies a requirement for molecules required for continued proliferation/initiation of DNA replication (see below). Similarly, exposure of lymphocytic lines to dexamethasone, a model agent for glucocorticoid-induced deletion of T cells/thymocytes, induces G1 arrest prior to morphological evidence of killing [Harmon et al., 1979].

The bursa of Fabricius, the primary site of B cell development in birds, contains a cohort of stem cells that gives rise to a rapidly proliferating population of lymphoblasts with IgM on their surface. Bursal stem cells and their progeny have been shown to be very sensitive to apoptosis induced by mechanical disruption of cell-cell contact in short term culture of bursal follicles as well as by γ radiation and other DNA damaging agents [Neiman et al., 1991]. Apoptosis is apparent within the first hour following tissue dispersion, and occurs primarily in S phase cells: fluorescence-activated cell sorting based on DNA content followed by agarose gel electrophoresis revealed that DNA degradation was occurring primarily in S phase cells. Apoptotic cells were positive for several molecular markers associated with S phase of the cell cycle [Neiman et al., 1994].

Since many growth factors act as progression factors [Pardee, 1989], promoting passage from G1 to S phase of the cell cycle, it is expected that apoptosis resulting from factor withdrawal correlates with arrest in G1. This is the case when ND7 cells, prepared by fusing rat dorsal root ganglion neurons with a neuroblastoma line, are transferred to serum-free medium [Howard et al., 1993] and when a variety of hematopoietic cell lines are deprived of particular interleukins [Nuñez et al., 1990; Colotta et al., 1992]. An exception to this generalization is the FVA cell line (murine erythroid hematopoietic progenitor cells infected with the anemia-inducing strain of Friend virus). These cells undergo apoptosis when deprived of erythropoietin [Kelley et al., 1992; Koury and Bondurant, 1990]. Cell loss occurs in the G1 and S phases of the cell cycle, but without apparent growth arrest [Kelley et al., 1994].

These data seem to place entry into apoptosis around the time of entry into S phase. Cells may halt abruptly in G1, without entering S, or may actually show evidence of having begun DNA replication. Since there are always exceptions to any generalization, we note that a human B cell lymphoma analogous to the murine WEHI-231 line seems to undergo activation-induced apoptosis from G2 rather than G1 [Ishigami et al., 1992].

2. Cell cycle arrest modifies the response to apoptosis-inducing agents. Cytotoxic lymphocytes (CTL) kill target cells by mechanisms that independently disrupt the cell membrane and induce apoptosis [Greenberg and Litchfield, in press; Squier and Cohen, 1994]. Inhibitors of replication fork-associated enzymes prevented DNA fragmentation and apoptosis in target cells, but not membrane lysis, suggesting that apoptosis was dependent on cell proliferation [Nishioka and Welsh, 1992]. In fact, target 3T3 fibroblasts could be made refractory to CTL-induced DNA fragmentation by serum deprivation. Infection of these quiescent 3T3 cells with herpes simplex virus 1 forced induction of DNA synthesis and restored susceptibility to CTL-induced apoptosis [Nishioka and Welsh, 1994].

Proliferating, mature T lymphocytes can be induced to undergo apoptosis after ligation of their antigen receptor [Boehme and Lenardo, 1993]. When proliferating A.E7 cells, a nontransformed CD4$^+$ T_H1 clone, were stimulated via the T cell receptor (TCR) by anti-CD3ϵ antibodies or via irradiated splenocytes and antigen, cells died by apoptosis. The extent of cell loss was directly proportional to the extent of proliferation. When A.E7 cells were incubated with cell cycle arrest agents prior to TCR ligation, cells that were blocked in G1 by mimosine, deferoxamine, or

dibutyryl cAMP were resistant to apoptosis, while cells arrested at G1/S by aphidicolin or in early S by excess thymidine were sensitized. G1 arrest did not simply select for a resistant population of cells, since release from arrest rapidly led to S phase and apoptosis. Like anti-IgM-mediated apoptosis of WEHI-231 cells, molecules acting in late G1/early S seem to be implicated in induction of apoptosis.

Another physiological inducer of apoptosis which has received careful cell cycle scrutiny is TNF-α. A variety of data indicate that TNF-α induces apoptosis in G1 in L929 cells [Darzynkiewicz et al., 1984; Kirstein et al., 1986; van de Loosdrecht et al., 1993]. However, G1 arrest with TGF-β, TNF-α, IL-1β, and IL-6 did not render cells more sensitive to TNF-α. Instead, cells were protected against TNF-α-induced cytotoxicity [Belizario and Dinarello, 1991]. This suggests that these agents may arrest cells at a point in G1 prior to the synthesis of TNF-sensitivity factors. Studies with human epithelial cells suggest that TNF sensitivity appears late in G1. Human HeLa cells are normally resistant to TNF-α, but, like numerous other cell types, can be sensitized to TNF-α by cotreatment with cycloheximide [Wallach, 1984], an agent which arrests HeLa cells late in G1, near the G1-S boundary. Cell cycle arrest in S phase with hydroxyurea was as effective at sensitizing HeLa cells as cycloheximide, suggesting a requirement for molecules acting at the time of the G1 to S transition in TNF-α-induced apoptosis [Meikrantz et al., 1994].

S phase arrest also potentiates apoptosis induced in HeLa cells by numerous agents with a wide variety of pharmacological activities, including staurosporine, 6-dimethylaminopurine, okadaic acid, caffeine, and γ-radiation [Meikrantz et al., 1994; Meikrantz and Schlegel, unpublished observations]. At the doses used, the majority of these agents have little or no effect on HeLa cell proliferation. In the case of staurosporine, arrest earlier in the cell cycle with lovastatin, which causes a reversible G1 arrest [Jakobisiak et al., 1991; Keyomarsi et al., 1991], did not potentiate apoptosis. Thus, a wide variety of apoptosis-inducing agents seem to require molecules synthesized in late G1/early S.

Apoptosis Induced by p53

Transfection of wild type p53 into transformed cells reduces colony forming ability [Baker et al., 1990; Isaacs et al., 1991; Johnson et al., 1991; Takahashi et al., 1992] and invariably leads to growth arrest in G1 [Diller et al., 1990; Martinez et al., 1991; Mercer et al., 1990; Michalovitz et al., 1990]. In murine myeloid leukemic lines, which typically lack p53, restoration of the wild type gene results in apoptosis [Ryan et al., 1993; Yonish-Rouach et al., 1991, 1993]. By transfecting M1 cells, a mouse myeloid leukemia line, or DP16-1 MEL cells, a Friend virus-induced murine erythroleukemia line, with a temperature-sensitive p53, cell cycle effects of p53-induced apoptosis were studied by shifting cells to the permissive temperature [Ryan et al., 1993; Yonish-Rouach et al., 1993]. In both cases, cells in G1 were preferentially susceptible to p53-induced killing. Treatment of the transfected M1 cells with IL-6, which arrests these cells in G1 [Resnitzsky and Kimchi, 1991; Resnitzsky et al., 1992], prevented apoptosis. However, TGF-β, another G1-arrest agent, failed to protect against p53-induced apoptosis; instead, in the presence of TGF-β, p53-dependent death was accelerated [Yonish-Rouach et al., 1993]. One explanation for these results is that IL-6 and TGF-β arrest at different points in G1, before and after synthesis of molecules required for p53-dependent apoptosis. Density-dependent growth arrest of transfected DP16-1 cells also blocked p53-dependent apoptosis, while cells released from the block died rapidly after shifting to the permissive temperature. Cells synchronized in G1 with mimosine or by isoleucine deprivation underwent apoptosis much more quickly than density-arrested cells. If arrested cells were released and allowed to progress out of G1 before shifting to the permissive temperature, apoptosis did not occur until cells had returned to G1 [Ryan et al., 1993]. The different response rates of density-arrested versus mimosine or isoleucine deprivation arrested cells again indicates that molecules required for p53-dependent apoptosis are synthesized at a later point in G1.

Apoptosis Induced by Perturbation of DNA Metabolism

Agents that block DNA replication, induce DNA damage, interfere with DNA topology, or block the segregation of chromosomes are lethal to the cell. Perhaps unsurprisingly, the majority of these agents have been found to induce apoptosis [see the tabulation in Sen and D'Incalci, 1992]. In some cases apoptosis occurred with little or no cell cycle specificity [Cotter et al., 1992]. In other cases, careful flow cytometric analysis revealed cell cycle differences in toxicity

and susceptibility. For example, HL-60 cells, a human promyelocytic leukemia line, were preferentially affected in G1 by nitrogen mustard; cells progressing through S phase were more susceptible to hydroxyurea, camptothecin, and teniposide; while G2/M cells preferentially underwent apoptosis in response to γ-radiation [Gorczyca et al., 1993]. These data raise two important points. First, it is possible that some agents directly activate apoptosis regardless of the cell cycle stage. Second, it is possible that cell cycle-related differences in response to such agents tells less about pathways of apoptosis induction than about the effect of the cell cycle stage on the severity of damage or the capacity to repair particular lesions [Gorczyca et al., 1993].

Apoptosis in Quiescent Cells

Several studies have reported apoptosis in quiescent cells. One such model system is involution of the prostate following androgen withdrawal, a process that has been studied extensively in the rat following castration. Within days of castration, the terminally differentiated secretory epithelial cells which line the ducts of the gland begin to undergo massive apoptosis. These cells, which compose as much as 85% of the cell population of the prostate, are lost within a week after castration [Lesser and Bruchovsky, 1973; Kerr and Searle, 1973], making this is an excellent tissue for studying the biochemistry and molecular biology of apoptosis. A number of apoptosis-specific proteins and "testosterone-repressed" messages have been identified [see Buttyan, 1991 for review]. Most interesting was the sequential induction of c-*fos* and c-*myc*, since induction of these genes, particularly in this sequence, is usually associated with the transition from quiescence to proliferative growth [Muller et al., 1984; Thompson et al., 1986], including regrowth of the prostate after androgen replacement [Katz et al., 1989]. This finding suggests that the quiescent acinar epithelial cells were being induced to reenter a defective cell cycle [Colombel et al., 1992] that ends in apoptosis rather than continuing DNA replication. In fact, cyclin A is expressed in regressing prostate, suggesting that these cells progress to a point quite late in G1 (R. Buttyan, personal communication). A similar early induction of c-*fos*, *jun*B, *jun*D, and c-*jun* is seen during involution of mammary epithelium at weaning [Marti et al., 1994] and during apoptosis induced by growth factor withdrawal from a myeloid leukemia line [Colotta et al., 1992].

Normally, c-*myc* is down-regulated during the transition from proliferative growth to quiescence; in fact, down regulation occurs in a cell cycle-independent manner as soon as mitogen is withdrawn [Dean et al., 1986; Waters et al., 1991]. When c-*myc* is expressed ectopically in Rat-1 fibroblasts or in primary rat embryo fibroblasts during serum withdrawal, proliferative growth is maintained and cells undergo apoptosis [Evan et al., 1992]. Similarly, apoptosis can be induced in serum-starved, quiescent Rat-1a fibroblasts following expression of *myc* from the Zn^{2+}-inducible metallothionein promoter [Hoang et al., 1994]. Since *myc* expression stimulates transcription (either directly or indirectly) of a number of genes acting late in G1 that are necessary for the G1 to S transition [Buchou et al., 1993; Hoang et al., 1994; Jansen-Dürr et al., 1993; Kim et al., 1994; Shibuya et al., 1992], ectopic expression of c-*myc* may force cells into a late G1-like state of preparedness for DNA replication or apoptosis.

A similar interpretation may apply to induction of apoptosis in quiescent cells by adenovirus E1A. Adenovirus E1A induces apoptosis when expressed in the absence of E1B 19K, a second adenovirus protein [Pilder et al., 1984; Rao et al., 1992; Subramanian et al., 1984; White et al., 1984a,b, 1992]. Infection of normal rat kidney cells with an adenovirus producing only the E1A protein and lacking the E1B region caused apoptosis only when the cells were growth arrested, either by growth to confluence or by serum starvation [Mymryk et al., 1994]. Like *myc*, E1A activates transcription of a number of genes required for DNA replication [Buchou et al., 1993; Moran, 1993; Nevins, 1992; Whyte et al., 1988]. In fact, E1A mutants that no longer promote DNA synthesis in rat kidney cells are also defective for inducing apoptosis [Howe et al., 1990].

In general, it appears that cells must progress to late G1 of the cell cycle for apoptosis to occur. Arrest prior to this stage delays or blocks apoptosis while arrest after this promotes apoptosis. What is significant about this particular moment in the cell cycle? First of all, this is the p53 restriction point. In normal cells, passage through this point of the cell cycle is at least partially regulated by p53, and it has become increasingly evident that many types of apoptosis-inducing agents are p53-dependent. What is

the role of p53 in apoptosis? How is the role of p53 in apoptosis related to its role as a cell cycle regulator? Secondly, this is the cell cycle stage often referred to as the "R point" in mammalian cells [Pardee, 1974] or "start" in yeast [Hartwell et al., 1974], the stage where cells become irreversibly committed to DNA replication. In organisms from yeast to man, passage through this point requires activation of specific CDKs. Are there restriction point-related CDKs whose activation is necessary for apoptosis to occur? We shall take up these questions in turn.

APOPTOSIS AND THE G1 RESTRICTION POINT: p53 AND p21

The idea that p53 acts as a "guardian of the genome" [Lane, 1992], monitoring a restriction point that requires cells to repair their DNA damage before entering S phase or else face apoptosis, is consistent with the finding that cells or cell lines derived from p53-deficient animals fail to undergo apoptosis in response to DNA damaging agents [Clarke et al., 1993, 1994; Lowe et al., 1993a,b; McCarthy et al., 1994; Merritt et al., 1994]. Loss of p53 function would then lead to fixation of genetic abnormalities resulting from DNA replication in the presence of DNA damage. If p53 were to act by eliminating cells with compromised DNA, the integrity of the organism would be maintained, although at the expense of cell loss due to apoptosis. Unlike the unicellular yeasts, which lack p53, this is a viable option for a multicellular organism. The proliferation of multiple malignancies in $p53^{-/-}$ mice could result from the persistent proliferation of cells with DNA damage. The ability of p53 to induce apoptosis on its own after re-introduction into p53-deficient cells, and its ability to limit the growth of existing neoplasms through apoptosis [Liu et al., 1994; Radinsky et al., 1994; Symonds et al., 1994], demonstrates a role for p53 in limiting neoplastic growth via induction of apoptosis. How p53 accomplishes its growth-limiting function must be related to its ties to the cell cycle and to control of CDK activation and inactivation, for it is via CDKs that the cell cycle progresses.

Cyclins are proteins whose levels rise and fall in an orderly way during the course of the cell cycle. They are positive regulatory subunits for the CDKs, conferring upon them substrate and cell cycle specificity. In addition to binding of the cyclin subunit, CDKs are regulated by activating and inactivating phosphorylations and by association of inhibitory CDK-interacting proteins (CIPs). These interactions are summarized schematically in Figure 1, which depicts an idealized CDK-cyclin complex. Events required to prepare the cell for entry into S phase are mediated by the D and E type cyclins [Sherr, 1994]. Principal among these events is phosphorylation of the product of the retinoblastoma susceptibility gene, pRB. Unphosphorylated pRB, present throughout most of G1, acts as a damper to cell proliferation by sequestering E2F transcription factors, which are necessary for transcription of genes required for entry into S phase. Phosphorylation of pRB by cyclin D- and cyclin E-associated CDKs releases the transcription factors and allows proliferation to advance. Ultimately, commencement of DNA replication requires activation of CDK-cyclin A complexes, which occurs at the time of the G1-S transition, a process described in more detail below.

Although identified (as *CIP1*) in a two-hybrid screen for proteins interacting with cdk2 [Harper et al., 1993] and purified (as CAP20) from mouse cells by virtue of its association with an inactive population of cdk2 [Gu et al., 1993], the p21 CIP has been shown to bind to and inactivate in vitro virtually every member of the CDK-cyclin family [Harper et al., 1993; Xiong et al., 1993]. Clues as to its physiological function come from how it was identified in two other systems. First, p21 was discovered as a senescent cell-derived inhibitor of DNA replication (*sdi1*) in human diploid fibroblasts by screening cDNAs prepared from senescent human diploid fibroblasts for their ability to inhibit [^{3}H]thymidine uptake upon transient overexpression in young, dividing fibroblasts [Noda et al., 1994]. Second, a connection to p53-dependent inhibition of growth was realized when p21 was discovered independently (under the name *WAF1*, for wild type p53-activated fragment 1) as a message whose transcription was entirely dependent on wild type p53 during p53-mediated growth arrest [El-Deiry et al., 1993]. p21 is induced in p53-dependent G1 arrest which follows γ irradiation of normal human diploid fibroblasts, resulting in inhibition of cdk2-cyclin E kinase activity, preventing phosphorylation of pRB, and thereby blocking entry into S phase [Dulič et al., 1994]. Similarly, in transformed cells (wild type p53-expressing colorectal carcinoma lines RKO and HCT-116), doxorubicin (Adriamycin) treatment induced p21 expression and blocked cdk2-cyclin E kinase activity. There was no induction of p21

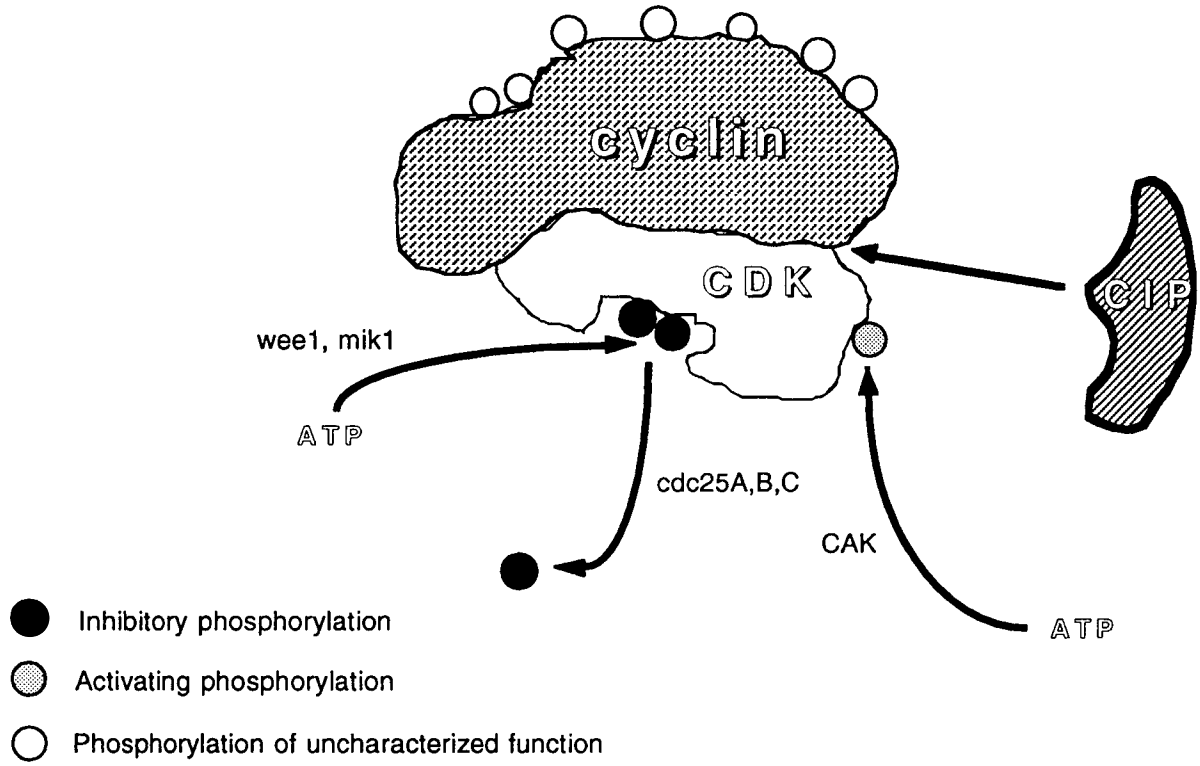

Fig. 1. Activation and inactivation of a model CDK-cyclin complex.

and little or no decrease in cyclin E-associated kinase activity following similar treatment of p53-deficient tumor lines treated with doxorubicin [El-Deiry et al., 1994]. While induction of p21 by serum- or growth factor-stimulation of quiescent cells [Noda et al., 1994] is independent of p53, induction of p21 by DNA damaging agents is completely dependent on p53 [Michieli et al., 1994]. Expression of p21 in a number of tumor-derived cell lines was able to suppress tumor growth. Expression of the p21 gene in the sense but not the antisense orientation resulted in a 10- to 20-fold decrease in colony-forming ability in p53-deficient tumor lines (the colon tumor line SW480, the brain tumor line DEL, and the lung adenocarcinoma line H1299 [El-Deiry et al., 1993]). Upon exposure to γ radiation, tumor cells containing wild type p53 induced p21 and underwent apoptosis, while p21 and apoptosis were lacking in p53-deficient cells [El-Deiry et al., 1994]. Thus, induction of p21 and consequent inhibition of CDKs may be a first step in p53-dependent apoptosis.

Interestingly, p53 and p21 also appear to induce a cell cycle arrest via mechanisms that are independent of CDK-cyclin complexes. SV40 DNA replication can be supported in an in vitro system containing T antigen and human DNA replication proteins [Waga and Stillman, 1994]. Addition of excess p21 to the reaction caused an accumulation of early DNA replication intermediates [Waga et al., 1994], suggesting that p21 was interfering with DNA elongation but not initiation/melting of origins of replication. Addition of the p16 CIP was without effect. The p21 inhibition could be overcome by addition of excess PCNA (proliferating cell nuclear antigen), a subunit required by DNA polymerase δ. This inhibition took place in the absence of detectable cyclins or CDKs, and involved formation of a complex between p21 and PCNA [Waga et al., 1994]. p53 itself physically interacts with a protein required for DNA replication, replication factor A (RPA, also referred to in the literature as RF-A or RP-A), a single-stranded DNA binding protein complex required for DNA replication [Fang and Newport, 1993; Waga and Stillman, 1994]. Binding of p53 to RPA blocks DNA replication [Dutta et al., 1993; He et al., 1993; Li and Botchan, 1993]. This may explain the observation that UV-induced apoptosis in a T antigen-immortalized pituitary (somatotropic) line expressing a temperature sensitive p53 mutant occurs in a p53-dependent manner (i.e., only at

the permissive temperature), but in the absence of p53-dependent transcription/translation (i.e., in the presence of actinomycin D or cycloheximide). Since shifting to the permissive temperature resulted in translocation of p53 to the nucleus, the authors propose that p53 affects apoptosis via direct participation in DNA repair or DNA degradation [Caelles et al., 1994].

Regardless of mechanism, the data strongly suggest that the first steps on the pathway of p53-dependent apoptosis are a cell cycle block at the beginning of S phase. In this light, it is interesting to note that cell cycle arrest is also a feature of several p53-independent pathways of apoptosis, for example, the activation-induced death of WEHI-231 cells, dexamethasone deletion of thymocytes, and TNF-α cytotoxicity. Furthermore, S phase arrest was able to promote apoptosis by p53-independent apoptosis-inducing agents in a T cell hybridoma [Boehme and Lenardo, 1993] and in HeLa cells, which are human papillomavirus positive and thus lack functional p53 [Meikrantz et al., 1994; Meikrantz and Schlegel, unpublished observations]. HeLa cells are normally very resistant to γ radiation-induced apoptosis. When HeLa cells are arrested in S phase, however, this resistance is overcome. We suggest that S phase arrest by agents such as hydroxyurea, thymidine, and aphidicolin chemically impose the cell cycle arrest normally mediated via p53. This immediately suggests that transient overexpression of p21—i.e., imposition of a molecular block to proliferation as opposed to a chemical one—will substitute for chemical agents in sensitizing cells to apoptosis inducers. Evidence that p21 is actually required for apoptosis comes from the observation that antisense constructs of p21 block p53-mediated apoptosis (J.C. Barrett, personal communciation).

Growth arrest by p53 cannot be the only trigger for apoptosis, as can be inferred from the different responses to γ radiation of transformed and nontransformed cells expressing wild type p53. Nontransformed cells typically show only growth arrest, while transformed cells undergo apoptosis [Dulič et al., 1994; El-Deiry et al., 1994]. Primary fibroblasts from $p53^{+/+}$ mice do not display a significant apoptotic response to γ radiation unless they are transformed by a combination of oncogenes (*E1A* and H-*ras*) [Lowe et al., 1993a]. These findings strongly suggest that an additional proliferation-associated step is required for apoptosis. Cell proliferation and passage through the G1 restriction point requires activation of specific CDKs, particularly cyclin E- and cyclin A-dependent protein kinases [Dou et al., 1993]. We have seen that inactivation of CDK-cyclin E complexes is associated with p53-dependent growth arrest and apoptosis; now we shall examine a role for activation of CDK-cyclin A complexes in apoptosis.

APOPTOSIS AND THE G1 RESTRICTION POINT: ACTIVATION OF CDKS

Activation of cdk2-cyclin A is required for the transition from G1 to S. Cdk2 becomes activated as a protein kinase at the time of entry into S phase [Rosenblatt et al., 1992]. Inactivation of cdk2 by microinjection of neutralizing antibodies to cyclin A [Pagano et al., 1992] or cdk2 [Pagano et al., 1993], treatment with antisense cyclin A nucleotides [Girard et al., 1991], or transfection of a dominant negative mutant of cdk2 [van den Heuvel and Harlow, 1993] prevents cells from entering S phase. Cdk2-cyclin A seems to affect DNA replication directly. Cdk2-cyclin A complexes are localized at foci of nuclear replication in terminally differentiated myotubes induced to re-enter S phase by transfection with T antigen [Cardoso et al., 1993], and phosphorylation of the p34 subunit of RPA is required for initiation and maintenance of DNA replication [Fang and Newport, 1993; Waga and Stillman, 1994]. Immunodepletion of cdk2 prevents DNA synthesis in a *Xenopus* cell free system [Fang and Newport, 1991] by preventing phosphorylation of the p34 subunit of RPA [Fang and Newport, 1993]. Like cdc2, cdk2 is subject to inactivating phosphorylations at the active site [Gu et al., 1992; Sebastian et al., 1993]. Cdk2 is dephosphorylated and activated by CDC25A: transcription of CDC25A begins in early G1 and peaks in late G1, and microinjection of anti-CDC25A antibodies blocks entry into S phase [Jinno et al., 1994].

Activation of cdk2-cyclin A appears to be the necessary, final step required for exit from G1 and commencement of DNA replication. Cyclin A is unique among the cyclins, being required at two points in the cell cycle: both for mitosis and for DNA replication [Girard et al., 1991; Pagano et al., 1992; Zindy et al., 1992]. In this light, it is interesting to note that apoptosis seems to depend on cell cycle processes acting at or about the time of the G1-S transition and comprises some of the same morphological changes found

in mitosis [Lazebnik et al., 1993; Rubin et al., 1993; Sen and D'Incalci, 1992; Ucker, 1991], the most prominent being chromatin condensation. Could activation of cyclin A-dependent protein kinases be the molecular link between apoptosis, the cell cycle, G1-S, and mitosis? Circumstantial evidence certainly seems to point in this direction. Arrest in the cell cycle prior to the appearance of cyclin A tends to protect cells from apoptosis [Belizario and Dinarello, 1991; Boehme and Lenardo, 1993; Colotta et al., 1992; Howard et al., 1993; Meikrantz et al., 1994; Nishioka and Welsh, 1994; Nuñez et al., 1990; Ryan et al., 1993; Yonish-Rouach et al., 1993]. This is not merely reflective of a requirement for p53 synthesis, since protection extends to p53-independent apoptosis-inducing agents as well (e.g., TNF-α, TCR ligation). Two oncogenes which induce apoptosis under restrictive growth conditions (i.e., in the absence of serum or without expression of a cooperating oncogene), c-*myc* and adenovirus E1A, are both transcriptional activators of the cyclin A gene [Buchou et al., 1993; Jansen-Dürr et al., 1993; Shibuya et al., 1992]. In the rat prostrate, cyclin A follows c-*myc* as one of the proliferation-associated genes whose transcription is enhanced during castration-induced apoptosis (R. Buttyan, personal communication). WEHI-231 cells accumulate increased levels of cyclin A protein following anti-IgM antibody treatment (Meikrantz, Boothby, and Schlegel, unpublished observations). Apoptosis due to prolonged S phase arrest (22 h) of the early $CD4^+$ T cell line AGF in high concentrations of thymidine is associated with translocation of cyclin A and cdc2 to the nucleus [Gazitt and Erdos, 1994]. Phosphorylation of the p34 subunit of RPA, which depends on cdk2 [Fang and Newport, 1993], is stimulated by exposure of cells to ionizing radiation [Liu and Weaver, 1993]. This suggests that cdk2-cyclin A activity may actually be stimulated by apoptosis-inducing agents. Finally, staurosporine, a known activator of CDKs, including cdk2-cyclin A [Meikrantz et al., 1994; Tam and Schlegel, 1992], is an acute activator of apoptosis in human cells [Jacobson et al., 1993; Meikrantz et al., 1994].

In S phase-arrested HeLa cells, apoptosis was induced by agents known to activate CDKs (caffeine, 6-dimethylaminopurine, staurosporine, and okadaic acid), with activation of cyclin A-dependent kinases occurring within 2 h after exposure [Meikrantz et al., 1994]. The extent of cyclin A-dependent kinase induction (two- to sevenfold) correlated well with the extent of apoptosis induction (15–80% of cells with apoptotic nuclei at 8 h following treatment). Where examined, both cdc2-cyclin A and cdk2-cyclin A were activated. These results raised the following question: since CDK-activating agents induced apoptosis, would other apoptosis-inducing agents activate CDKs? This was found to be the case with TNF-α, which has previously been reported to act synergistically with chemical CDK activators such as caffeine [Belizario et al., 1993] and staurosporine [Beyaert et al., 1993]. HeLa cells are normally resistant to TNF-α, unless it is given simultaneously with cycloheximide [Wallach, 1984] or to S phase-arrested cells [Meikrantz et al., 1994]. In hydroxyurea-arrested cells, TNF-α stimulated CDK-cyclin A activity about threefold. Cotreatment with TNF-α and cycloheximide increased kinase activity about sevenfold. Interestingly, cycloheximide alone induced a fivefold increase in CDK-cyclin A activity, suggesting a mechanism for its potentiation of TNF-α killing. HeLa cells in this model system had to be *arrested* in S phase in order to undergo apoptosis: cells passing synchronously through S phase were not sensitized to killing. We suggest that this system is a "chemical" model for apoptosis: S phase arrest by hydroxyurea chemically imposes the cell cycle arrest component, while TNF-α or chemical treatment provides the signal(s) for CDK activation.

Induced transcription of cyclin A followed by CDK-cyclin A activation also takes place in serum-starved Rat 1a fibroblasts during *myc*-induced apoptosis [Hoang et al., 1994]. Two types of analysis were carried out to study cyclin expression in this system. First, cell lines were created in which myc was constitutively overexpressed. In these cells, serum withdrawal led to extensive apoptosis, whereas serum withdrawal from cultures of parental cells had little effect. Analysis of cyclin transcription in the myc-overexpressing cells showed that cyclin A transcription was elevated compared with parental Rat 1a cells, while transcription of cyclin B, cyclin C, cyclin D1, and cyclin E were unaffected. A second group of cell lines was then created, expressing cyclin A under control of the Zn^{2+}-inducible metallothionein promoter. Serum withdrawal plus exposure to 50 μM Zn^{2+} led to apoptosis. Not all of the cells expressed cyclin A to the same level following Zn^{2+} induction, and the extent of apoptosis observed in low serum plus Zn^{2+} conditions indicated a dose-dependent induction of apoptosis by cyclin A. Thus, in the

presence of growth arrest by serum starvation, expression of cyclin A alone was sufficient to induce apoptosis.

Is CDK-cyclin A activation required for apoptosis? The ability of a wide variety of apoptosis-inducing agents to induce CDK-cyclin A activity in S phase-arrested HeLa cells is suggestive of a common requirement for this enzyme, and the sufficiency of cyclin A induction alone to promote apoptosis in serum-starved fibroblasts is further evidence for such a mechanism. However, the most direct evidence for a requirement for CDK activity in apoptosis comes from studies of cytolysis induced by fragmentin-2/granzyme B, a protease component of the lytic granules released by cytotoxic T cells or natural killer cells during destruction of their targets [reviewed in Greenberg and Litchfield, in press]. Fragmentin-2/granzyme B rapidly (within a few hours) induced apoptosis when added to detergent-permeabilized cells. Premature activation of cdc2 was found to be required for fragmentin-2/granzyme B-induced apoptosis [Shi et al., 1994]. Cdc2 was rapidly activated at the initiation of apoptosis, and DNA fragmentation and nuclear condensation could be prevented by an excess of cdc2 peptide substrate. At least part of the induced kinase activity has subsequently been found to be associated with cyclin A (A. Greenberg, personal communication). Most importantly, fragmentin-2/granzyme B-induced apoptosis was temperature dependent in FT210 cells, which are homozygous for a temperature sensitive cdc2 allele [Th'ng et al., 1990]. At the restrictive temperature, where mutant cdc2 is rapidly degraded, fragmentin-2/granzyme B-induced apoptosis was inhibited by as much as 50%. This strongly implicates CDK-cyclin A activation as a necessary step in apoptosis induction. Although the maximum inhibition seen was 50%, we note that FT210 cells contain a significant amount of cdk2-cyclin A that is unaffected by shift to the cdc2-restrictive temperature [Hamaguchi et al., 1992]. Since both cdc2-cyclin A and cdk2-cyclin A are activated in HeLa cells undergoing apoptosis, it is possible that elimination of all cyclin A-dependent kinase activity might inhibit apoptosis to an even greater degree. The relative predominance of cdc2-cyclin A complexes versus cdk2-cyclin A complexes early in the cell cycle seems to vary from cell type to cell type and as a function of growth conditions. For example, significant differences between monolayer and suspension HeLa cultures have been reported [Elledge et al., 1992]. Recently, we have found that transient expression of dominant negative mutants of cdc2 and cdk2 both suppress TNF-α-induced apoptosis in HeLa cells, although cdk2-cyclin A seems to be the crucial complex mediating apoptosis in these monolayer cells [Meikrantz and Schlegel, manuscript in preparation].

Much has been made in the literature of the morphological similarities between mitotic catastrophe in yeast, premature mitosis in mammalian cells, and apoptosis. It has been suggested that apoptosis is a premature, abortive mitosis. At the beginning of this review, we presented the conceptual similarity between apoptosis and mitotic catastrophe in yeast, relating the two processes to checkpoint controls that impinge on CDKs. However, some clear biochemical distinctions can be made between the two processes. First and most importantly, cdc2-cyclin B, whose activation is absolutely required for mitosis [Nurse, 1990] and premature mitosis [Steinmann et al., 1991] is not activated during apoptosis in HeLa cells [Meikrantz et al., 1994]. Instead, activation of CDK-cyclin A complexes is observed. We note that cyclin A has no clear counterpart among the yeast cyclins. The chromatin morphology induced by premature activation of cdc2-cyclin B is different from that induced in apoptosis: chromosome spreads prepared from apoptotic cells stain homogeneously, typically containing one or more nonstaining "holes" [Arends et al., 1990; Meikrantz et al., 1994], and do not present the characteristic "pulverized" appearance of premature chromatin condensation [Rao and Johnson, 1970]. In a cell free system that mimicks many of the morphological characteristics of apoptosis [Lazebnik et al., 1993], depletion of cdc2-cyclin B did not suppress the response, mitosis-specific phosphorylation of the MPM-2 proteins was not detected, and the nuclear lamins were not phosphorylated. In fact, the nuclear lamina disassembly that was seen seems to have been due to proteolysis rather than phosphorylation of the lamin proteins.

Although we have suggested that apoptosis correspond may to the cyclin A-dependent half of mitosis [Meikrantz et al., 1994], it could equally be viewed as a premature and aberrant entry into S phase, resulting in condensation and cleavage of chromatin rather than DNA replication (see Fig. 2). Thus, while agents like staurosporine induce premature mitosis in G2-arrested cells, they induce apoptosis in G1/S-arrested cells. In this sense, apoptosis resembles

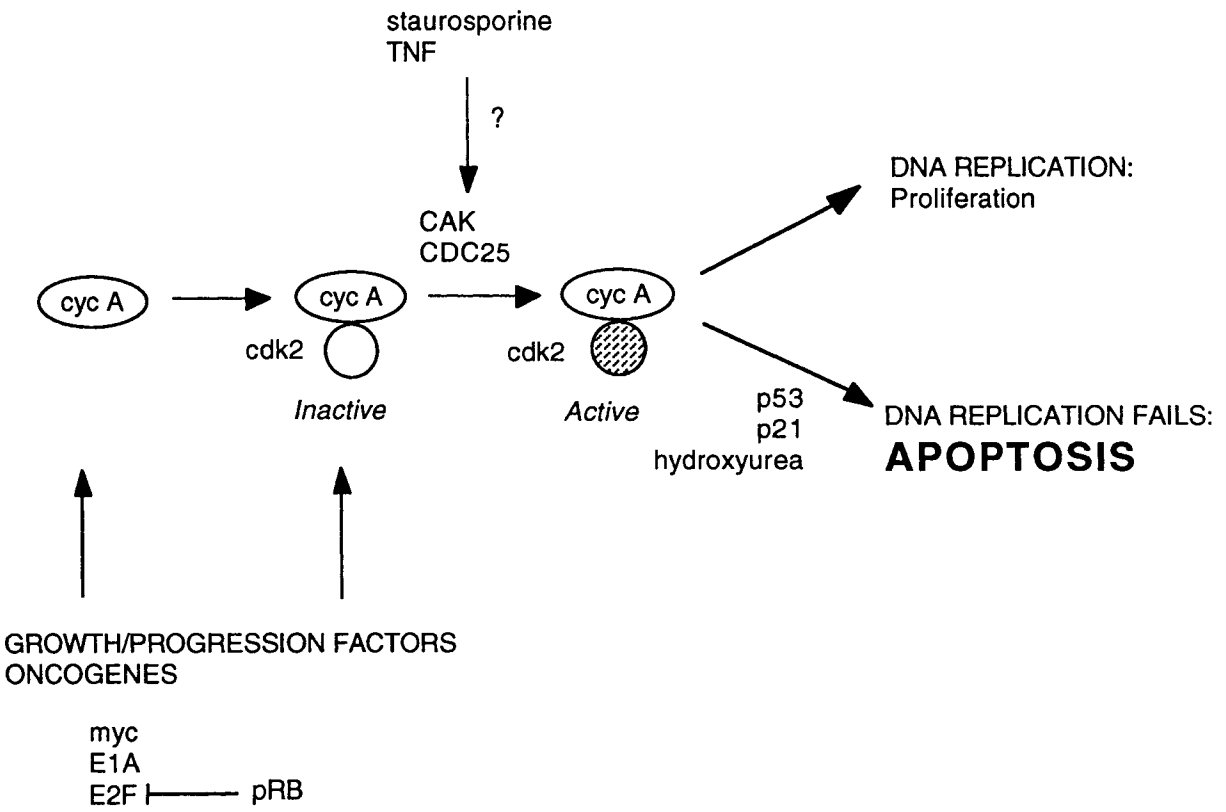

Fig. 2. Relationship of CDK-cyclin A activation to DNA replication and apoptosis.

mitotic catastrophe: both events are characterized by improper timing of CDK activation and by uncoupling the normal interdependence of cell cycle events.

REFERENCES

Allan DJ, Harmon BV, Kerr JFR (1987): Cell death in spermatogenesis. In Potten CS (ed): "Perspectives on Mammalian Cell Death." New York: Oxford University Press, pp 229–258.

Arends MJ, Morris RG, Wyllie AH (1990): Apoptosis: the role of the endonuclease. Am J Pathol 136:593–608.

Ashwell JD, Cunningham RE, Noguchi PD, Hernandez D (1987): Cell growth cycle block of T cell hybridomas upon activation with antigen. J Exp Med 165:173–194.

Baker SJ, Markowitz S, Fearon ER, Willson JK, Vogelstein B (1990): Suppression of human colorectal carcinoma cell growth by wild-type *p53*. Science 249:912–915.

Belizario JE, Dinarello CA (1991): Interleukin 1, interleukin 6, tumor necrosis factor and transforming growth factor β increase cell resistance to tumor necrosis factor cytotoxicity by growth arrest in the G1 phase of the cell cycle. Cancer Res 51:2379–2385.

Belizario JE, Tilly JL, Sherwood SW (1993): Caffeine potentiates the lethality of tumour necrosis factor in cancer cells. Br J Cancer 67:1229–1235.

Benhamou LE, Cazenave PA, Sarthou P (1990): Anti-immunoglobulins induce death by apoptosis in WEHI-231 B lymphoma cells. Eur J Immunol 20:1405–1407.

Beyaert R, Vanhaesebroeck B, Heyninck K, Boone E, Valck DD, Schulze-Osthoff K, Haegeman G, Van Roy F, Fiers W (1993): Sensitization of tumor cells to tumor necrosis factor action by the protein kinase inhibitor staurosporine. Cancer Res 53:2623–2630.

Bishop CJ, Whiting VA (1983): The role of natural killer cells in the intravascular death of intravenously injected murine tumour cells. Br J Cancer 48:441–444.

Boehme SA, Lenardo MJ (1993): Propriocidal apoptosis of mature T lymphocytes occurs at S phase of the cell cycle. Eur J Immunol 23:1552–1560.

Boyd A, Schrader J (1981): The regulation of growth and differentiation of a murine B cell lymphoma. II. The inhibition of WEHI 231 by anti-immunoglobulin antibodies. J Immunol 126:2466–2469.

Buchou T, Kranenburg O, van Dam H, Roelen D, Zantema A, Hall FL, van der Eb A (1993): Increased cyclin A and decreased cyclin D levels in adenovirus 5 E1A-transformed rodent cell lines. Oncogene 8:1765–1773.

Bursch W, Taper HS, Lauer B, Schulte-Hermann R (1985): Quantitative histological and histochemical studies on the occurrence and stages of controlled cell death (apoptosis) during regression of rat liver hyperplasia. Virchows Arch [B] 50:153–166.

Buttyan R (1991): Genetic response of prostate cells to androgen deprivation: insights into the cellular mechanisms of apoptosis. In Tomei LD, Cope FO (eds): "Apoptosis: The Molecular Basis of Cell Death." Plainview, NY: Cold Spring Harbor Laboratory Press, pp 157–173.

Buttyan R, Zakeri Z, Lockshin R, Wolgemuth D (1988): Cascade induction of c-*fos*, c-*myc*, and heat shock 70K transcripts during regression of the rat ventral prostate gland. Mol Endocrinol 2:650–657.

Caelles C, Heimberg A, Karin M (1994): p53-dependent apoptosis in the absence of transcriptional activation of p53-target genes. Nature 370:220–223.

Cardoso MC, Leonhardt H, Nadal-Ginard B (1993): Reversal of terminal differentiation and control of DNA replication—cyclin A and cdk2 specifically localize at subnuclear sites of DNA replication. Cell 74:979–992.

Clarke A, Gledhill S, Hooper M, Bird C, Wyllie A (1994): p53 dependence of early apoptotic and proliferative responses within the mouse intestinal epithelium following gamma-irradiation. Oncogene 9:1767–1773.

Clarke AR, Purdie CA, Harrison DJ, Morris RG, Bird CC, Hooper ML, Wyllie AH (1993): Thymocyte apoptosis induced by p53-dependent and independent pathways. Nature 362:849–852.

Cohen JJ (1991): Programmed cell death in the immune system. Adv Immunol 50:55–85.

Colombel M, Olsson CA, Ng P-Y, Buttyan R (1992): Hormone-regulated apoptosis results from reentry of differentiated prostate cells onto a defective cell cycle. Cancer Res 52:4313–4319.

Colotta F, Polentarutti N, Sironi M, Mantovani A (1992): Expression and involvement of c-*fos* and c-*jun* protooncogenes in programmed cell death induced by growth factor deprivation in lymphoid cell lines. J Biol Chem 267:18278–18283.

Columbano A, Ledda-Columbano GM, Coni P, Faa G, Liguori C, SantaCruz G, Pani P (1985): Occurrence of cell death (apoptosis) during involution of liver hyperplasia. Lab Invest 52:670–675.

Cotter TG, Glynn JM, Echeverri F, Green DR (1992): The induction of apoptosis by chemotherapeutic agents occurs in all phases of the cell cycle. Anticancer Res 12:773–780.

Darzynkiewicz Z, Williamson B, Carswell EA, Old LJ (1984): The cell cycle specific effects of tumour necrosis factor. Cancer Res 44:83–90.

Dean M, Levine RA, Ran W, Kindy MS, Sonenshein GE, Campisi J (1986): Regulation of c-*myc* transcription and mRNA abundance by serum growth factors and cell contact. J Biol Chem 261:9161–9166.

Diller L, Kassel J, Nelson CE, Gryka MA, Litwak G, Gebhardt M, Bressac B, Ozturk M, Baker SJ, Vogelstein B, Friend SH (1990): p53 functions as a cell cycle control protein in osteosarcomas. Mol Cell Biol 10:5772–5781.

Dou QP, Levin AH, Zhao S, Pardee AB (1993): Cyclin E and cyclin A as candidates for the restriction point protein. Cancer Res 53:1493–1497.

Duke RC (1991): Apoptosis in cell-mediated immunity. In Tomei LD, Cope FO (eds): "Apoptosis: The Molecular Basis of Cell Death." Plainview, NY: Cold Spring Harbor Laboratory Press, pp 209–226.

Dulič V, Kaufmann W, Wilson S, Tlsty T, Lees E, Harper J, Elledge S, Reed S (1994): p53-dependent inhibition of cyclin-dependent kinase activities in human fibroblasts during radiation-induced G1 arrest. Cell 76:1013–1023.

Dutta A, Ruppert JM, Aster JC, Winchester E (1993): Inhibition of DNA replication factor RPA by p53. Nature 365:79–82.

El-Deiry WS, Harper JW, O'Connor PM, Velculescu VE, Canman CE, Jackman J, Pietenpol JA, Burrell M, Hill DE, Wang Y, Wiman KG, Mercer WE, Kastan MB, Kohn KW, Elledge SJ, Kinzler KW, Vogelstein B (1994): *WAF1/CIP1* is induced in *p53*-mediated G1 arrest and apoptosis. Cancer Res 54:1169–1174.

El-Deiry WS, Tokino T, Velculescu VE, Levy DB, Parsons R, Trent JM, Lin D, Mercer WE, Kinzler KW, Vogelstein B (1993): *WAF1*, a potential mediator of p53 tumor suppression. Cell 75:817–825.

Elledge SJ, Richman R, Hall FL, Williams RT, Lodgson N, Harper JW (1992): CDK2 encodes a 33-kDa cyclin A-associated protein kinase and is expressed before CDC2 in the cell cycle. Proc Natl Acad Sci U S A 89:2907–2911.

Evan GI, Wyllie AH, Gilbert CS, Littlewood TD, Land H, Brooks M, Waters CM, Penn LZ, Hancock DC (1992): Induction of apoptosis in fibroblasts by c-myc protein. Cell 69:119–128.

Fang F, Newport J (1991): Evidence that the G1-S and G2-M transitions are controlled by different Cdc2 proteins in higher eukaryotes. Cell 66:731–742.

Fang F, Newport JW (1993): Distinct roles of cdk2 and cdc2 in RP-A phosphorylation during the cell cycle. J Cell Sci 106:983–994.

Gazitt Y, Erdos GW (1994): Fluctuations and ultrastructural localization of oncoproteins and cell cycle regulatory proteins during growth and apoptosis of synchronized AGF cells. Cancer Res 54:950–956.

Gerschenson LE, Rotello RJ (1991): Apoptosis and cell proliferation are terms of the growth equation. In Tomei LD, Cope FO (eds): "Apoptosis: The Molecular Basis of Cell Death." Plainview, NY: Cold Spring Harbor Laboratory Press, 175–192.

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

Glucksmann A (1951): Cell deaths in normal vertebrate ontogeny. Biol Rev 26:59–86.

Gorczyca W, Gong J, Ardelt B, Traganos F, Darzynkiewicz Z (1993): The cell cycle related differences in susceptibility of HL-60 cells to apoptosis induced by various antitumor agents. Cancer Res 53:3186–3192.

Green DR, Scott DW (1994): Activation-induced apoptosis in lymphocytes. Curr Opin Immunol 6:476–487.

Greenberg AH, Litchfield DW (in press): Granzymes and apoptosis: targeting the cell cycle. Curr Opin Microbiol Immunol.

Gu Y, Rosenblatt J, Morgan DO (1992): Cell cycle regulation of CDK2 activity by phosphorylation of Thr160 and Tyr15. EMBO J 11:3995–4005.

Gu Y, Turck CW, Morgan DO (1993): Inhibition of CDK2 activity *in vivo* by an associated 20K regulatory subunit. Nature 366:707–710.

Hamaguchi JR, Tobey RA, Pines J, Crissman HA, Hunter T, Bradbury EM (1992): Requirement for $p34^{cdc2}$ kinase is restricted to mitosis in the mammalian *cdc2* mutant FT210. J Cell Biol 117:1041–1053.

Harmon JM, Norman MR, Fowlkes BJ, Thompson EB (1979): Dexamethasone induces irreversible G1 arrest and death of a human lymphoid cell line. J Cell Physiol 98:267–278.

Harper JW, Adami GR, Wei N, Keyomarsi K, Elledge SJ (1993): The p21 Cdk-interacting protein Cip1 is a potent inhibitor of G1 cyclin-dependent kinases. Cell 75:805–816.

Hartwell LH, Culotti J, Pringle JR, Reid BJ (1974): Genetic control of cell division cycle in yeast. Science 183:46–51.

Hasbold J, Klaus GGB (1990): Anti-immunoglobulin antibodies induced apoptosis in immature B cell lymphomas. Eur J Immunol 20:1685–1690.

He Z, Brinton BT, Greenblatt J, Hassell JA, Ingles CJ (1993): The transactivator proteins VP16 and GAL4 bind replication factor A. Cell 73:1223–1232.

Hoang AT, Cohen KJ, Barrett JF, Bergstrom DA, Dang CV (1994): Participation of cyclin A in Myc-induced apoptosis. Proc Natl Acad Sci U S A 91:6875–6879.

Howard MK, Burke LC, Mailhos C, Pizzey A, Gilbert CS, Lawson WD, Collins MKL, Thomas NSB, Latchman DS (1993): Cell cycle arrest of proliferating neuronal cells by serum deprivation can result in either apoptosis or differentiation. J Neurochem 60:1783–1791.

Howe JA, Mymryk JS, Egan C, Branton PE, Bayley ST (1990): Retinoblastoma growth suppressor and a 300-kDa protein appear to regulate cellular DNA synthesis. Proc Natl Acad Sci U S A 87:5883–5887.

Isaacs BW, Carter BS, Ewing CM (1991): Wild-type p53 suppresses growth of human prostate cancer cells containing mutant p53 alleles. Cancer Res 51:4716–4720.

Ishigami T, Kim K-M, Horiguchi Y, Higaki Y, Hata D, Heike T, Katamura K, Mayumi M, Mikawa H (1992): Anti-IgM antibody-induced cell death in a human B lymphoma cell line B104 represents a novel programmed cell death. J Immunol 148:360–368.

Jacobson MD, Burne JF, King MP, Miyashita T, Reed JC, Raff MC (1993): Bcl-2 blocks apoptosis in cells lacking mitochondrial DNA. Nature 361:365–369.

Jakobisiak M, Bruno S, Skierski JS, Darzynkiewicz Z (1991): Cell cycle-specific effects of lovastatin. Proc Natl Acad Sci USA 88:3628–3632.

Jansen-Dürr P, Meichle A, Steiner P, Pagano M, Finke K, Botz J, Wessbecher J, Draetta G, Eilers M (1993): Differential modulation of cyclin gene expression by *MYC*. Proc Natl Acad Sci U S A 90:3685–3689.

Jinno S, Suto K, Nagata A, Igarashi M, Kanaoka Y, Nojima H, Okayama H (1994): Cdc25A is a novel phosphatase functioning early in the cell cycle. EMBO J 13:1549–1556.

Johnson P, Gray D, Mowat M, Benchimol S (1991): Expression of wild-type p53 is not compatible with continued growth of p53-negative tumor cells. Mol Cell Biol 11:1–11.

Katz AE, Benson MC, Wise GJ, Olsson CA, Bandyk MG, Sawczuk IS, Tomashefsky P, Buttyan R (1989): Gene activity during the early phase of androgen-stimulated rat prostate regrowth. Cancer Res 49:5889–5894.

Kelley LL, Green WF, Hicks GG, Bondurant MC, Koury MJ, Ruley HE (1994): Apoptosis in erythroid progenitors deprived of erythropoietin occurs during the G1 and S phases of the cell cycle without growth arrest or stabilization of wild-type p53. Mol Cell Biol 14:4183–4192.

Kelley LL, Koury MJ, Bondurant MC (1992): Regulation of programmed death in erythroid progenitor cells by erythropoietin: effects of calcium and of protein and RNA synthesis. J Cell Physiol 151:487–496.

Kerr JFR, Harmon BV (1991): Definition and incidence of apoptosis: an historical perspective, In Tomei LD, Cope F (eds): "Apoptosis: The Molecular Basis of Death." Plainview, NY: Cold Spring Harbor Press, pp 5–29.

Kerr JFR, Searle J (1973): Deletion of cells by apoptosis during castration-induced involution of the rat prostate. Virchows Arch B Cell Pathol 13:87–102.

Kerr JFR, Searle J, Harmon BV, Bishop CJ (1987): Apoptosis In Potten CS (ed): "Perspectives on Mammalian Cell Death." New York: Oxford University Press, pp 93–178.

Kerr JFR, Wyllie AH, Currie AR (1972): Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. Br J Cancer 26:239–257.

Keyomarsi K, Sandoval L, Band V, Pardee AB (1991): Synchronization of tumor and normal cells from G1 to multiple cell cycles by lovastatin. Cancer Res 51:3602–3609.

Kim YH, Buchholz MA, Chrest FJ, Nordin AA (1994): Up-regulation of *c-myc* induces the gene expression of the murine homologues of $p34^{cdc2}$ and cyclin-dependent kinase-2 in T lymphocytes. J Immunol 152:4328–4335.

Kirstein M, Fiers W, Baglioni C (1986): Growth inhibition and cytotoxicity of tumor necrosis factor in L-929 cells is enhanced by high cell density and inhibition of mRNA synthesis. J Immunol 137:2277–2280.

Koury MJ, Bondurant MC (1990): Erythropoietin retards DNA breakdown and prevents programmed death in erythroid progenitor cells. Science 248:378–381.

Lane DP (1992): p53, guardian of the genome. Nature 358:15–16.

Lazebnik YA, Cole S, Cooke CA, Nelson WG, Earnshaw WC (1993): Nuclear events of apoptosis *in vitro* in cell-free mitotic extracts: a model system for analysis of the active phase of apoptosis. J Cell Biol 123:7–22.

Ledda-Columbano GM, Columbano A (1991): Apoptosis and hepatocarcinogenesis. In Tomei LD, Cope FO (eds): "Apoptosis: The Molecular Basis of Cell Death." Plainview, NY: Cold Spring Harbor Laboratory Press, pp 101–119.

Lesser B, Bruchovsky N (1973): The effects of testosterone, 5-α-dihydrotestosterone and adenosine 3′,5′-monophosphate on cell proliferation and differentiation in rat prostate. Biochim Biophys Acta 308:426–437.

Li R, Botchan MR (1993): The acidic transcriptional activation domains of VP16 and p53 bind the cellular replication protein A and stimulate *in vitro* BPV-1 DNA replication. Cell 73:1207–1221.

Liu T, Zhang W, Taylor D, Roth J, Goepfert H, Clayman G (1994): Growth suppression of human head and neck cancer cells by the introduction of a wild-type p53 gene via a recombinant adenovirus. Cancer Res 54:3662–3667.

Liu VF, Weaver DT (1993): The ionizing radiation-induced replication protein-A phosphorylation response differs between ataxia-telangiectasia and normal human cells. Mol Cell Biol 13:7222–7231.

Lowe SW, Ruley HE, Jacks T, Housman DE (1993a): p53-dependent apoptosis modulates the cytotoxicity of anticancer agents. Cell 74:957–967.

Lowe SW, Schmitt EM, Smith SW, Osborne BA, Jacks T (1993b): p53 is required for radiation-induced apoptosis in mouse thymocytes. Nature 362:847–849.

Maheswaran S, McCormack JE, Sonenshein GE (1991): Changes in phosphorylation of *myc* oncogene and *RB* antioncogene protein products during growth arrest of the murine lymphoma WEHI 231 cell line. Oncogene 6:1965–1971.

Marti A, Jehn B, Costello E, Keon N, Ke G, Martin F, Jaggi R (1994): Protein kinase A and AP-1 (c-Fos/JunD) are induced during apoptosis of mouse mammary epithelial cells. Oncogene 9:1213–1223.

Martinez J, Georgoff I, Martinez J, Levine AJ (1991): Cellular localization and cell cycle regulation by a temperature sensitive p53 protein. Genes Dev 5:151–159.

McCarthy SA, Symonds HS, Van Dyke T (1994): Regulation of apoptosis in transgenic mice by simian virus 40 T antigen-mediated inactivation of p53. Proc Natl Acad Sci U S A 91:3979–3983.

Meikrantz W, Gisselbrecht S, Tam SW, Schlegel R (1994): Activation of cyclin A-dependent protein kinases during apoptosis. Proc Natl Acad Sci USA 91:3754–3758.

Mercer WE, Shields MT, Amin M, Sauve MGJ, Apella E, Romano JW, Ullrich SJ (1990): Negative growth regulation in a glioblastoma cell line that conditionally expresses human wild-type p53. Proc Natl Acad Sci USA 87:6166–6170.

Merritt A, Potten C, Kemp C, Hickman J, Balmain A, Lane D, Hall P (1994): The role of p53 in spontaneous and radiation-induced apoptosis in the gastrointestinal tract of normal and p53-deficient mice. Cancer Res 54:614–617.

Michalovitz D, Halevy O, Oren M (1990): Conditional inhibition of transformation and of cell proliferation by a temperature-sensitive mutant of p53. Cell 62:671–680.

Michieli P, Chedid M, Lin D, Pierce JH, Mercer WE, Givol D (1994): Induction of *WAF1/CIP1* by a p53-independent pathway. Cancer Res 54:3391–3395.

Moore JV (1987): Death of cells and necrosis of tumours, In Potten CS (ed): "Perspectives on Mammalian Cell Death." New York: Oxford University Press, pp 295–325.

Moran E (1993): Interaction of adenoviral proteins with pRB and p53. FASEB J 7:880–885.

Muller R, Bravo C, Burkhardt I, Curran T (1984): Induction of c-*fos* gene and protein by growth factors precedes the activation of c-*myc*. Nature 314:546–548.

Mymryk JS, Shire K, Bayley ST (1994): Induction of apoptosis by adenovirus type 5 E1A in rat cells requires a proliferation block. Oncogene 9:1187–1193.

Neiman PE, Blish C, Heydt C, Loring G, Thomas SJ (1994): Loss of cell cycle controls in apoptotic lymphoblasts of the bursa of Fabricius. Mol Biol Cell 5:763–772.

Neiman PE, Thomas SJ, Loring G (1991): Induction of apoptosis during normal and neoplastic development of the bursa of Fabricius. Proc Natl Acad Sci U S A 88:5857–5861.

Nevins JR (1992): E2F: a link between the Rb tumor suppressor protein and viral oncoproteins. Science 258:424–429.

Nishioka WK, Welsh RM (1992): Inhibition of cytotoxic T lymphocyte-induced target cell DNA fragmentation, but not lysis, by inhibitors of DNA topoisomerases I and II. J Exp Med 175:23–27.

Nishioka WK, Welsh RM (1994): Susceptibility to cytotoxic T lymphocyte-induced apoptosis is a function of the proliferative status of the target. J Exp Med 179:769–774.

Noda A, Ning Y, Venable SF, Pereira-Smith OM, Smith JR (1994): Cloning of senescent cell-derived inhibitors of DNA synthesis using an expression screen. Exp Cell Res 211:90–98.

Novack DV, Korsmeyer SJ (1994): Bcl-2 protein expression during murine development. Am J Pathol 145:61–73.

Nuñez G, London L, Hockenbery D, Alexander M, McKearn JP, Korsmeyer SP (1990): Deregulated *bcl-2* gene expression selectively prolongs survival of growth factor-deprived hemopoietic cell lines. J Immunol 144:3602–3610.

Nurse P (1990): Universal control mechanism regulating onset of M-phase. Nature 344:503–508.

O'Shea JD, Hay MF, Cran DG (1978): Ultrastructural changes in the theca interna during follicular atresia in sheep. J Reprod Fertil 54:183–187.

Otsuki Y, Misaki O, Sugimoto O, Ito Y, Tsujimoto Y, Akao Y (1994): Cyclic *bcl-2* gene expression in human uterne endometrium during menstrual cycle. Lancet 344:28–29.

Pagano M, Pepperkok R, Lukas J, Baldin B, Ansorge W, Bartek J, Draetta G (1993): Regulation of the cell cycle by the cdk2 protein kinase in cultured human fibroblasts. J Cell Biol 121:101–111.

Pagano M, Pepperkok R, Verde F, Ansorge W, Draetta G (1992): Cyclin A is required at two points in the human cell cycle. EMBO J 11:961–971.

Pardee AB (1974): A restriction point for control of normal animal cell proliferation. Proc Natl Acad Sci U S A 71: 1286–1290.

Pardee AB (1989): G1 events and regulation of cell proliferation. Science 246:603–608.

Pilder S, Logan J, Shenk T (1984): Deletion of the gene encoding the adenovirus 5 early region 1B—21,000-molecular weight polypeptide leads to degradation of viral and cellular DNA. J Virol 52:664–671.

Potten CS (1977): Extreme sensitivity of some intestinal crypt cells to X and γ irradiation. Nature 269:518–521.

Radinsky R, Fidler I, Price J, Esumi N, Tsan R, Petty M, Bucana C, Bareli M (1994): Terminal differentiation and apoptosis in experimental lung metastases of human osteogenic sarcoma cells by wild type p53. Oncogene 9:1877–1883.

Raff MC (1992): Social controls on cell survival and cell death. Nature 356:397–399.

Rao L, Debbas M, Sabbatini P, Hockenbery D, Korsmeyer S, White E (1992): The adenovirus E1A proteins induce apoptosis which is inhibited by the E1B 19-kDa and Bcl-2 proteins. Proc Natl Acad Sci U S A 89:7742–7746.

Rao PN, Johnson RT (1970): Mammalian cell fusion: studies on the regulation of DNA synthesis and mitosis. Nature 225:159–164.

Resnitzsky D, Kimchi A (1991): Deregulated c-myc expression abrogates the interferon and interleukin-6 mediated G0/G1 cell cycle arrest but not other inhibitory responses in M1 myeloblastic cells. Cell Growth Differ 2:33–41.

Resnitzsky D, Tiefenbrun N, Berissi H, Kimchi A (1992): Interferons and interleukin 6 suppress phosphorylation of the retinoblastoma protein in growth-sensitive hematopoietic cells. Proc Natl Acad Sci U S A 89:402–406.

Rosenblatt J, Gu Y, Morgan D (1992): Human cyclin-dependent kinase 2 (CDK2) is activated during the S and G2 phase of the cell cycle and associates with cyclin A. Proc Natl Acad Sci U S A 89:2824–2828.

Rubin LL, Philpott KL, Brooks SF (1993): The cell cycle and cell death. Curr Biol 3:391–394.

Ryan JJ, Danish R, Gottlieb CA, Clarke MF (1993): Cell cycle analysis of p53-induced cell death in murine erythroleukemia cells. Mol Cell Biol 13:711–719.

Sandow BA, West NB, Norman RL, Brenner RM (1979): Hormonal control of apoptosis in hamster uterine luminal epithelium. Am J Anat 156:15–35.

Saunders JW (1966): Death in embryonic systems. Science 154:604–612.

Scott DW, Livnat D, Pennell C, Keng P (1986): Lymphoma models for B cell activation and tolerance. III. Cell cycle dependence for negative signaling of WEHI-231 B lymphoma cells by anti-μ. J Exp Med 164:156–164.

Scott DW, Tuttle J, Livnat D, Haynes W, Cogswell JP, Keng P (1985): Lymphoma models for B-cell activation and tolerance. II. Growth inhibition by anti-μ of WEHI-231 and the selection and properties of resistant mutants. Cell Immunol 93:124–131.

Sebastian B, Kakizuka A, Hunter T (1993): Cdc25M2 activation of cyclin-dependent kinases by dephosphorylation of threonine-14 and tyrosine-15. Proc Natl Acad Sci U S A 90:3521–3524.

Sen S, D'Incalci M (1992): Apoptosis: biochemical events and relevance to cancer chemotherapy. FEBS Lett 307: 122–127.

Sherr C (1994): G1 phase progression: Cycling on cue. Cell 79:551–555.

Shi L, Nishioka WK, Th'ng J, Bradbury EM, Litchfield DW, Greenberg AH (1994): Premature p34*cdc2* activation required for apoptosis. Science 263:1143–1145.

Shi Y, Glynn JM, Guilbert LJ, Cotter TG, Bissonnette RP, Green DR (1992): Role for c-myc in activation-induced apoptotic cell death in T cell hybridomas. Science 257:212–214.

Shibuya H, Yoneyama M, Ninomiya-Tsuji J, Matsumoto K, Taniguchi T (1992): IL-2 and EGF receptors stimulate the hematopoietic cell cycle via different signaling pathways: demonstration of a novel role for c-*myc*. Cell 70:57–67.

Squier MKT, Cohen JJ (1994): Cell-mediated cytotoxic *mechanisms*. Curr Opin Immunol 6:447–452.

Steinmann KE, Belinsky GS, Lee D, Schlegel R (1991): Chemically induced premature mitosis: differential response in rodent and human cells and the relationship to cyclin B synthesis and $p34^{cdc2}$/cyclin B complex formation. Proc Natl Acad Sci U S A 88:6843–6847.

Subramanian T, Kuppuswamy M, Gysbers J, Mak S, Chinnadurai G (1984): 19-kDa tumor antigen coded by early region E1b of adenovirus 2 is required for efficient synthesis and for protection of viral DNA. J Biol Chem 259: 11777–11783.

Swartzendruber DC, Congdon CC (1963): Electron microscope observations on tingible body macrophages in mouse spleen. J Cell Biol 19:641–646.

Symonds H, Krall L, Remington L, Saenz-Robles M, Lowe S, Jacks T, Van Dyke T (1994): p53-dependent apoptosis suppresses tumor growth and progression in vivo. Cell 78:703–711.

Takahashi T, Carbone D, Takahashi T, Nau MM, Hida T, Linnoila I, Ueda R, Minna JD (1992): Wild-type but not mutant p53 suppresses the growth of human lung cancer cells bearing multiple genetic lesions. Cancer Res 52:2340–2343.

Tam S, Schlegel R (1992): Staurosporine overrides checkpoints for mitotic onset in BHK cells. Cell Growth Differ 3:811–817.

Th'ng JPH, Wright PS, Hamaguchi J, Lee MG, Norbury CJ, Nurse P, Bradbury EM (1990): The FT210 cell line is a mouse G2 phase mutant with a temperature-sensitive *CDC2* product. Cell 63:313–324.

Thompson NL, Meade JE, Braun L, Boyetter M, Shank PR, Fausto N (1986): Sequential protooncogene expression during rat liver regeneration. Cancer Res 46:3111–3117.

Ucker DS (1991): Death by suicide: one way to go in mammalian cellular development? New Biologist 3:103–109.

Umansky SR (1991): Apoptotic process in the radiation-induced death of lymphocytes. In Tomei LD, Cope FO (eds): "Apoptosis: The Molecular Basis of Cell Death." Plainview, NY: Cold Spring Harbor Laboratory Press, pp 193–208.

van de Loosdrecht AA, Ossenkoppele GJ, Beelen RJJ, Broekhoven MG, Langenhuijsen MMAC (1993): Cell cycle specific effects of tumor necrosis factor α in monocyte mediated leukemic cell death and the role of β_2 integrins. Cancer Res 53:4399–4407.

van den Heuvel S, Harlow E (1993): Distinct roles for cyclin-dependent kinases in cell cycle control. Science 262:2050–2054.

Waga S, Hannon GJ, Beach D, Stillman B (1994): The p21 inhibitor of cyclin-dependent kinases controls DNA replication by interaction with PCNA. Nature 369:574–577.

Waga S, Stillman B (1994): Anatomy of a DNA replication fork revealed by reconstitution of SV40 DNA replication *in vitro*. Nature 369:207–212.

Walker NI, Bennett RE, Kerr JFR (1989): Cell death by apoptosis during involution of the lactating breast in mice and rats. Am J Anat 185:19–32.

Wallach D (1984): Preparations of lymphotoxin induce resistance to their own cytotoxic effect. J Immunol 132:2464–2469.

Warner GL, Ludlow JW, Nelson DA, Gaur A, Scott DW (1992): Anti-immunoglobulin treatment of murine B cell lymphomas induces active transforming growth factor β but pRB hypophosphorylation is transforming growth factor β independent. Cell Growth Differ 3:175–181.

Waters C, Littlewood T, Hancock D, Moore J, Evan G (1991): c-*myc* protein expression in untransformed fibroblasts. Oncogene 6:101–109.

White E, Blose SH, Stillman B (1984a): Nuclear envelope localization of an adenovirus tumor antigen maintains the integrity of cellular DNA. Mol Cell Biol 4:2865–2875.

White E, Grozdzicker T, Stillman BW (1984b): Mutations in the gene encoding the adenovirus E1B 19K tumor antigen cause degradation of chromosomal DNA. J Virol 52:410–419.

White E, Sabbatini P, Debbas M, Wold WSM, Kusher DI, Gooding LR (1992): The 19-kilodalton adenovirus E1B transforming protein inhibits programmed cell death and prevents cytolysis by tumor necrosis factor α. Mol Cell Biol 12:2570–2580.

Whyte P, Buchkovich KJ, Horowitz JM, Friend SH, Raybuck M, Weinberg RA, Harlow E (1988): Association between an oncogene and an anti-oncogene: the adenovirus E1A proteins bind to the retinoblastoma gene product. Nature 334:124–126.

Wyllie AH (1980): Glucocorticoid-induced thymocyte apoptosis is associated with endogenous endonuclease activation. Nature 284:555–556.

Wyllie AH, Kerr JFR, Currie AR (1980): Cell death: the significance of apoptosis. Int Rev Cytol 68:251–306.

Wyllie AH, Kerr JFR, Currie AR (1973a): Cell death in the normal neonatal rat adrenal cortex. J Pathol 111:255–261.

Wyllie AH, Kerr JFR, Macaskill IAM, Currie AR (1973b): Adrenocortical cell deletion: the role of ACTH. J Pathol 111:85–94.

Xiong X, Hannon GJ, Zhang H, Casso D, Kobayashi R, Beach D (1993): p21 is a universal inhibitor of cyclin kinases. Nature 366:701–704.

Yonish-Rouach E, Grunwald D, Wilder S, Kimchi A, May E, Lawrence J-J, May P, Oren M (1993): p53-mediated cell death: relationship to cell cycle control. Mol Cell Biol 13:1415–1423.

Yonish-Rouach E, Resnitzky D, Lotem J, Sachs L, Kimchi A, Oren M (1991): Wild-type p53 induces apoptosis of myeloid leukaemic cells that is inhibited by interleukin-6. Nature 352:345–347.

Yoshizato K (1989): Biochemistry and cell biology of amphibian metamorphosis with a special emphasis on the mechanism of removal of larval organs. Int Rev Cytol 119:97–149.

Yuan J, Horvitz HR (1990): The *Caenorhabditis elegans* genes *ced-3* and *ced-4* act cell autonomously to cause programmed cell death. Dev Biol 138:33–41.

Zindy F, Lamas E, Chenivese X, Sobczak J, Wang J, Fesquet D, Henglein B, Brechot C (1992): Cyclin A is required in S phase in normal epithelial cells. Biochem Biophys Res Commun 182:1144–1154.