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Cell Cycle and Apoptosis[1]

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Abstract

In multicellular organisms, cell proliferation and death must be regulated to maintain tissue homeostasis. Many observations suggest that this regulation may be achieved, in part, by coupling the process of cell cycle progression and programmed cell death by using and controlling a shared set of factors. An argument in favor of a link between the cell cycle and apoptosis arises from the accumulated evidence that manipulation of the cell cycle may either prevent or induce an apoptotic response. This linkage has been recognized for tumor suppressor genes such as *p53* and *RB*, the dominant oncogene, c-*Myc*, and several cyclin-dependent kinases (Cdks) and their regulators. These proteins that function in proliferative pathways may also act to sensitize cells to apoptosis. Indeed, unregulated cell proliferation can result in pathologic conditions including neoplasias if it is not countered by the appropriate cell death. Translating the knowledge gained by studying the connection between cell death and cell proliferation may aid in identifying novel therapies to circumvent disease progression or improve clinical outcome. *Neoplasia* (2000) **2,** 291–299.

Keywords: apoptosis, cell cycle, c-*myc*, *p53*, pRb, Cdks.

Introduction

Apoptosis is a highly conserved mechanism by which eucaryotic cells commit suicide. It enables an organism to eliminate unwanted and defective cells through an orderly process of cellular disintegration that has the advantage of not inducing an undesirable inflammatory response [1]. Apoptotic elimination of cells occurs during normal development and turnover, as well as in a variety of pathological conditions. Indeed, improper regulation of apoptosis contributes to disorders such as cancer, viral infection, autoimmune diseases, neurodegenerative disorders, stroke, anemia and AIDS [2].

Apoptosis can be triggered by a wide variety of signals. These include Fas ligand, tumor necrosis factor, growth factor withdrawal, viral or bacterial infection, oncogenes, irradiation, ceramide, and chemotherapeutic drugs [2]. Often, these signals are cell-type-specific. Even if these agents vary from cell to cell, there is basic biochemical machinery underlying the process of regulated cell death. The morphological changes characteristic of the apoptotic process are mainly due to caspases, a family of cysteine proteases that act as effectors of the cell death pathway [3]. Their activation leads to the cleavage of specific proteins that include lamins, topoisomerases, DNA-dependent protein kinase (DNA-PK), poly(ADP-ribose) polymerase (PARP) and some cell cycle regulators.

The cell cycle is a conserved mechanism by which eucaryotic cells replicate themselves. In metazoans, the process of cell loss and cell gain must be homeostatically balanced in order to generate and maintain the complex architecture of tissues, and also to allow adaptation to changing circumstances. One way in which this connection may be achieved is through the coupling of the cell cycle and programmed cell death, perhaps by using or controlling a shared a set of factors [4].

A direct link between apoptosis and the cell cycle may be supposed from noting that mitosis and apoptosis display very similar morphological features. During both processes, cells lose substrate attachment, become rounded, shrink, condense their chromatin and display membrane blebbing. Although mitosis and apoptosis share some features, of course there are critical differences. For example, in apoptotic cells, DNA is degraded at internucleosomal linker sites, yielding DNA fragments in multiples of 180 bp resulting in a nucleosomal ladder. In addition, during apoptosis, cell membrane proteins are cross-linked, making the membrane more rigid [2], and apoptotic cells are typically phagocytosed by adjacent cells or macrophages. In contrast, during mitosis, DNA is segregated and the cell is divided by cytokinesis, resulting in two healthy, viable daughter cells.

A more solid argument in favor of a link between the cell cycle and apoptosis is based on several instances in which apoptosis is regulated by genes that are involved in cell cycle progression. There is accumulating evidence that manipulation of the cell cycle may prevent or induce an apoptotic response depending upon the cellular context [5]. Because cellular context is crucial, these proteins may serve to tie cell death to proliferative signals even though they may not be part of the cell's apoptotic machinery. It is possible that these proliferative proteins act to sensitize cells to apoptosis.

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This review, after briefly describing cell cycle regulation, summarizes the role of cell proliferation regulators in apoptosis. Specifically, we will discuss *p53*, Myc and pRb, players with important relevance in tumor progression. We will also describe the involvement of cyclin-dependent kinases (Cdks) and their regulators in apoptosis.

Cell Cycle Machinery

The cell cycle is a set of events responsible for cell duplication [6]. Transmission of genetic information from one cell generation to the next requires genome replication during the S-phase, and its segregation to the two new daughter cells during mitosis or M-phase. S- and M-phases are crucial events rigorously ordered in a cyclic process that allows for the correct duplication of the cell without accumulating genetic abnormalities. In a normal cell cycle, S-phase is always proceeded by M-phase and M-phase does not occur until S-phase is complete. Between the S- and M-phases, there are two preparatory gaps. G1 separates M from S, and G2 is between S and M. When the cell undergoes differentiation, it exits from the G1 phase of the cell cycle to enter into a quiescent state referred to as G0.

The timing and order of cell cycle events are monitored during cell cycle checkpoints that occur at the G1/S boundary, in S-phase, and during the G2/M-phases [6]. The checkpoints are a series of control systems enabling proliferation only in the presence of stimulatory signals such as growth factors. They also contribute to the fidelity with which genetic information is passed from one generation to the next. The checkpoints also are activated by DNA damage and by mis-aligned chromosomes at the mitotic spindle. In this case, the growth arrest caused by checkpoints allows the cell to repair the damage. After damage repair, progression through the cell cycle resumes. If the damage cannot be repaired, the cell is eliminated through apoptosis.

Progression of the eucaryotic cell through the four phases of the cell cycle is mediated by sequential activation and inactivation of Cdks (Figure 1). The Cdks belong to a well-conserved family of serine/threonine protein kinases. Their kinase activity is dependent on the presence of activating subunits called cyclins. The abundance of specific cyclins increases during the phase of the cell cycle in which they are required and decreases during phases in which they are not needed. Cyclin D associates with Cdk4 and Cdk6 during early G1. The primary target of the G1 kinases is the pRb family of proteins. Their phosphorylation permits the transcription of genes necessary for S-phase. The cyclin D/Cdk complexes are crucial to the cell cycle by coupling extracellular signals to the cell cycle [6]. In fact, upon mitogenic stimulation, cyclin D/Cdk complexes are activated

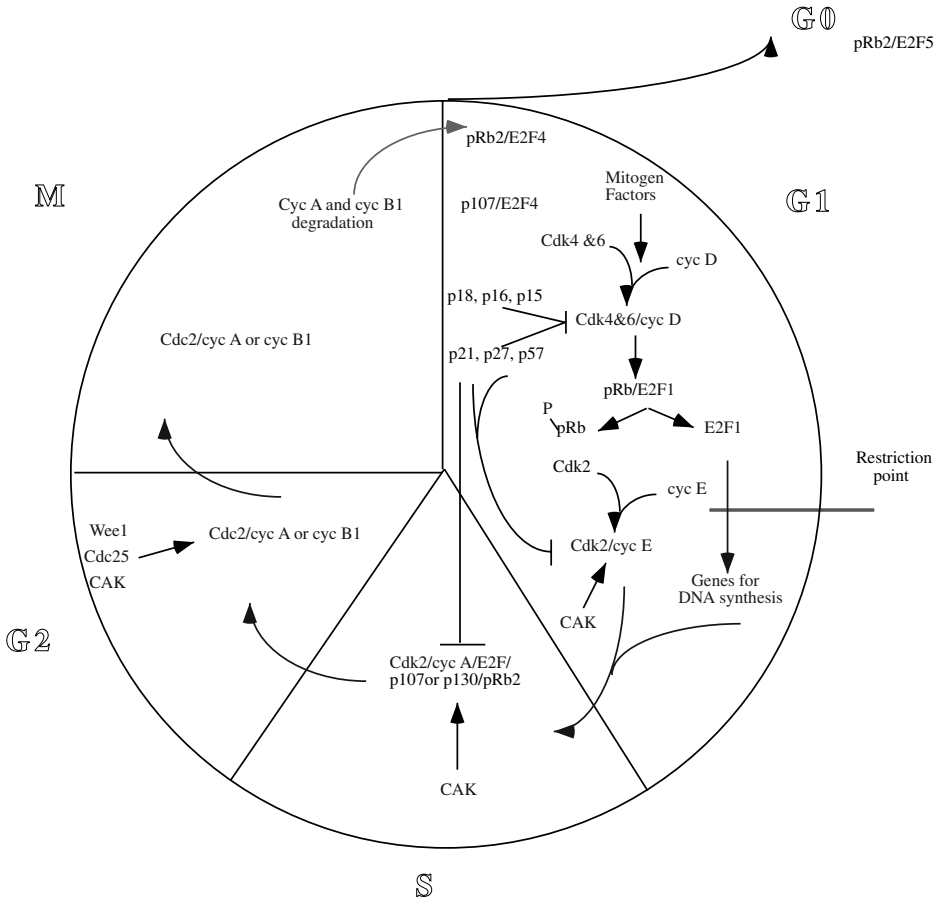

Figure 1. *Schematic representation of the relationship between Cdks and Cdk regulators during the cell cycle.*

and the cells progress from G0 into G1. In late G1, after the restriction point (R-point) is passed, the cell is committed to entering S-phase and cyclin D–Cdk activity is no longer required for cell cycle progression. Cyclin E activates Cdk2 during the G1-to-S-phase transition. Cyclin A binds to Cdk2 during S-phase or to Cdc2 in the G2-to-M-phase transition. The cyclin B/Cdc2 complex also functions during the G2-to-M-phase transition.

The activity of Cdk complexes is also regulated by other mechanisms. Their activation requires association with their cyclin partner and phosphorylation by Cdk-activating kinase (CAK). In addition, Cdk activity is suppressed by phosphorylation at conserved threonine and tyrosine residues. Dephosphorylation of these residues and consequent Cdk activation are mediated by the Cdc25 phosphatase family. Another mechanism regulating the Cdk activity is mediated by the Cdk-inhibitory subunits (CKIs). In mammalian cells, two classes of CKIs, the Cip/Kip and the Ink4 families, provide a tissue-specific mechanism by which cell cycle progression can be restrained in response to extracellular and intracellular signals [7]. The Cip/Kip family includes $p21^{Cip1}$, $p27^{Kip1}$ and $p57^{Kip2}$ and predominantly inhibits the Cdks of the G1-to-S-phase transition. The Ink4 (inhibitors of Cdk4) family contains four members, $p15^{Ink4b}$ $p16^{Ink4}$, $p18^{Ink4c}$ and $p19^{Ink4d}$, several of which are mutated or deleted in certain types of human cancers.

pRb, the retinoblastoma protein, is a negative regulator of cell growth and is a tumor suppressor. It is mutated or deleted in many cancers, such as retinoblastoma and carcinomas of the lung, breast, bladder, bone and prostate [8]. It is the principal of a family of proteins that also encompasses pRb2/p130 and p107 [9]. These proteins have overlapping, but distinct, activities in regulating the cell cycle. In general, hypophosphorylated forms of these three proteins are functionally active in blocking transcription of S-phase genes. This ability depends on their capacity to bind and actively repress the E2F factors [10]. The E2F factors are transcriptional activators composed of an E2F protein and a member of the DP family of proteins [11]. In this review, these complexes are referred simply as E2F. E2F positively regulates the transcription of S-phase genes. Upon mitogen stimulation, G1 Cdks phosphorylate pRb and pRb-like proteins [6], thereby releasing the E2F complexes and allowing expression of genes for DNA synthesis.

c-*myc* and the Dual Signal Model

The c-*myc* proto-oncogene has been studied extensively and its product is the best characterized member of the Myc family of short-lived nuclear phosphoproteins [12]. It encodes a protein that functions as a transcription factor that stimulates cell proliferation. Myc's activity depends upon association with Max, and this association is required for mitogenesis [12]. Myc is induced by mitogens in proliferating cells and is absent in quiescent cells. When Myc is ectopically expressed, it drives cells into the cell cycle. Conversely, inhibition of c-*myc* expression leads to growth arrest [13].

A connection between Myc and apoptosis has also been demonstrated. Myc overexpression promotes apoptosis during serum deprivation or hypoxia [14]. Moreover, anti–apoptotic genes, such as Bcl-2, suppress Myc-induced apoptosis [15]. The mitogenic and pro–apoptotic functions of Myc both require dimerization with Max and sequence specific DNA binding [12]. It is most likely that Myc–Max dimers execute their functions by regulating specific target genes.

Several models have been proposed to explain the apparently contradictory roles of Myc in both proliferation and apoptosis. It is tempting to speculate that inappropriate Myc expression pushes cells into a cell cycle during serum deprivation or hypoxia for which they were not prepared, thereby sensitizing cells to apoptosis. However, Myc-induced apoptosis is independent of cell cycle position and so this explanation is not satisfying [5]. The "dual signal" model has been postulated in which Myc activates genes involved in both proliferation and apoptotic pathways [5]. Mitogens stimulate Myc's proliferation pathway, while anti–apoptotic factors, such as Bcl-2, may shut down Myc's apoptotic pathway (Figure 2). The fact that Myc-induced apoptosis, but not proliferation, is inhibited by Bcl-2 suggests that there are two distinct sets of genes involved in these pathways that can be modulated by different signals [15]. In addition, Myc apoptosis does not require the synthesis of novel polypeptides, indicating that the Myc-induced apoptotic pathway is "set" in growing cells but that it is being suppressed.

The precise mechanism by which Myc induces apoptosis has not been demonstrated. However, Cdc25A is a well-established transcriptional target of Myc [16]. Cdc25A mediates Myc's effect on the cell cycle by inducing Cdk activity. Cdc25A also induces apoptosis in serum-deprived fibroblasts [16], identical to what has been observed for Myc. Inhibition of Cdc25A expression by antisense also diminishes the ability of Myc to induce apoptosis. Therefore, it is possible that Cdc25A mediates Myc's apoptotic effect; however, additional research is required to determine how Cdc25A, in turn, induces apoptosis.

p53: Growth Arrest or Apoptosis?

The importance of *p53* in normal cell growth is supported by the fact that its function is lost in approximately half of all human cancers. It is involved in several different aspects of cell cycle arrest, apoptosis, control of genome integrity, and DNA repair [17] (Figure 3). It regulates a variety of processes by transactivating genes that are involved in different cellular functions (e.g. p21, Gadd45, Mdm2, Egfr, PCNA, cyclin D1, cyclin G, TGFα, 14-3-3σ, Bax, Bcl-XL, Fas1, FasL, *DR5* and Igf-BP3). *p53* also can inhibit the expression of specific genes, such as topoisomerase IIa.

p53 is a nuclear DNA-binding phosphoprotein that exists normally as a tetramer able to bind specific DNA sequences. A variety of stimuli causes its activation by increasing the protein's half-life and the rate of translational initiation of its mRNA [18]. Posttranslational modification of the protein, as

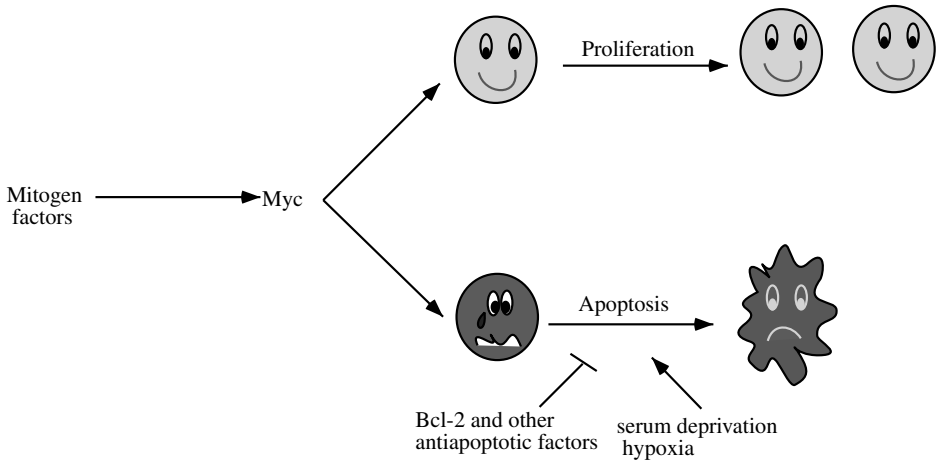

Figure 2. *The "Dual Signal Model". c-Myc induces proliferation or apoptosis depending on the presence or the absence of anti–apoptotic regulators.*

well as alternative splicing and binding of regulatory proteins, also are involved in activating *p53* [19].

p53 influences proliferation by acting predominately in the G1 phase of the cell cycle progression. Oncogenic and hyperproliferative stimuli (e.g. Myc, Ras, E1A), DNA damage by UV, hypoxia, γ-irradiation, nucleotide deprivation or chemotherapeutic drugs activate *p53* [17]. Activated *p53* causes a G1 arrest by inducing expression of p21 and the consequent inhibition of cyclin D/Cdks. In these conditions, pRb is not phosphorylated and cells do not progress through the G1-to-S-phase transition. Schneider *et al.* [20] reported that *p53* also can regulate CAK activity leading to Cdk2 downregulation. These findings imply a direct involvement of *p53* in triggering growth arrest by its interaction with the CAK complex without the need of Cdk inhibitors.

Numerous studies demonstrate that *p53* also functions at the G2/M checkpoint [19]. The ability of *p53* to induce a G2 arrest is cell-type-specific. Quite frequently in many mouse, rat and human cell lines overexpression of *p53* inhibits entry into mitosis [21]. This checkpoint seems to be activated when DNA synthesis is blocked and prevents segregation of damaged or incompletely synthesized DNA. The mechanism behind this G2/M growth arrest still is unknown. Probably, *p53* induces a G2 arrest by decreasing cyclin B1 transcription and synthesis [22]. *p53* may induce a G2 arrest also thorough 14-3-3σ. 14-3-3σ inactivates Cdc25C and, consequently, Cdc2 activity [23].

p53 is also involved in the spindle checkpoint that blocks re-replication of DNA when the mitotic spindle has been damaged, by inhibiting entry into S-phase [24]. In addition, *p53* plays a role in controlling centrosome duplication. In fact, it has been observed that *p53* directly associates with centrosomes [25] and prevents mitotic failure regulating the number of centrosomes [26].

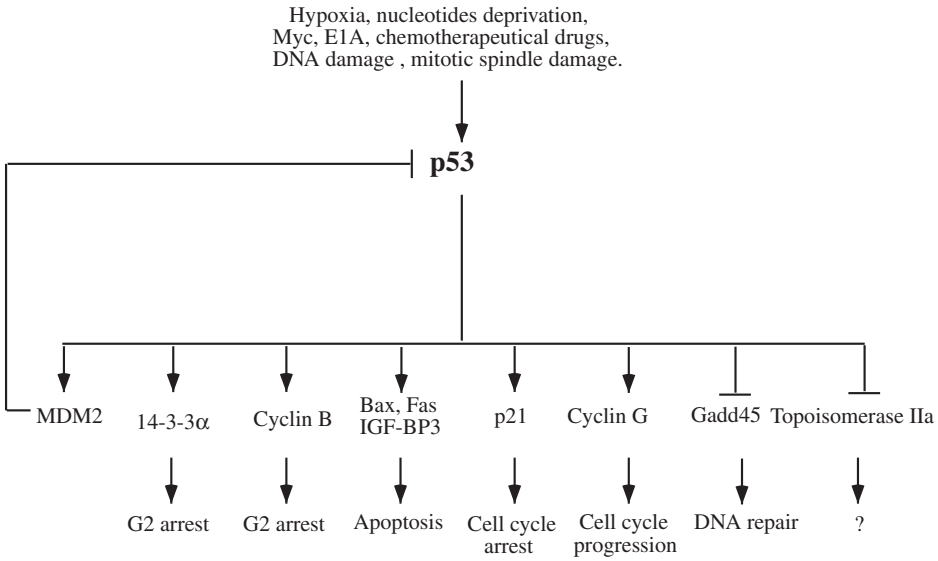

Figure 3. *p53 pathways. Several factors induce p53 activity. p53 controls G1 and G2 checkpoints activating or inhibiting genes involved in cell cycle, apoptosis and DNA repair.*

p53 plays an important role in triggering apoptosis in certain cell types. *p53*-dependent apoptosis is induced by DNA damage, hypoxia, withdrawal of growth factors, or expression of Myc or E1A [17]. In most cases, *p53*-induced apoptosis appears to be independent of its transcriptional function because it occurs in the presence of protein synthesis inhibitors and because *p53* mutants unable to transactivate can still induce apoptosis [27]. *p53* also represses the transcription of specific genes that inhibit its capability to induce apoptosis such as Bcl-2 [28]. Protein–protein interaction between *p53* and factors involved in the DNA repair mechanism can account for additional ways by which *p53* induces apoptosis without transcription activation. One example is the interaction of *p53* with TFIIH, a transcription factor involved in DNA repair [29].

However, in some systems, the transactivation function of *p53* plays an important role in inducing apoptosis. For example, the pro–apoptotic proteins, Bax and IgF-Bp3, are transcriptional targets of *p53*. *p53* also transactivates Fas transcription [30]. Fas is a cell surface protein that triggers apoptosis upon ligand (FasL) binding. Fas belongs to the *TNFR* family of genes coding for membrane receptors, and are involved in the regulation of cell proliferation. In the presence of DNA damage, *p53* transactivates also the *DR5* gene [31]. *DR5* is another member of *TNFR* family. Like Fas, the binding of the ligand (TRAIL) to DR5 activates caspase-dependent apoptosis. The induction of Fas and DR5 transcription by *p53* indicates that the *p53* transcriptional function may assist in modulating the apoptotic response triggered by certain stimuli.

After stimulation, *p53* may induce cell death or cell cycle arrest; the different outcome depends upon a variety of variables. The genetic background of the cell can be important. For instance, p21-null cells do not arrest in response to DNA damage but proceed to apoptose in a *p53*-dependent manner [32]. Impaired cross-talk between *p53* and pRb can lead to the same result (see below). The anti–apoptotic effect of Bcl-2 and adenoviral E1B can prevent *p53*-mediated apoptosis [33,34]. In some hematopoietic cells, the presence of specific interleukins (ILs) can direct the cell towards apoptosis or cell arrest after *p53* induction [35]. The extent of DNA damage and *p53* protein levels are also factors that contribute to making the choice between life and death. It may be that during *p53*-induced cell cycle arrest, the cell attempts to repair damage, perhaps with the assistance of its enhanced repair capacity from *p53* induction of Gadd45. If the damage is too extensive to be repaired, the cell then is committed to die.

It has been shown that *p53* can be regulated by oncogenic and hyperproliferative stimuli. CKI p14 and its murine counterpart p19, coded by the ARF/INK4 locus, are the main players in this mechanism. Mitogenic signals derived by oncogenes such as Myc, E1A or E2F are able to induce p14 synthesis. Subsequently, p14 binds Mdm2 inhibiting its capability to induce *p53* degradation [for review, see Ref. [36]]. Indirect stabilization of *p53* by p14 results in cell death.

pRb

pRb is not only a growth suppressor but also an anti–apoptotic factor. *In vivo* studies show that pRb knockout mice die *in utero* at 14 to 15 days [37]. They have defects in the haematopoietic system and have impaired development of the central and the peripheral nervous system because of massive cell death. Inappropriate cell death also is present in the liver, lens, and skeletal muscle precursors.

In vitro experiments confirm results obtained with transgenic mice. TGF-β1 causes cell death by suppressing pRb expression and phosphorylation [38]. IFNγ-induced apoptosis is blocked in pRb-positive cells [39]. Restoring the function of pRb in a pRb-null osteosarcoma cell line inhibits irradiation-induced apoptosis [40]. The presence of HPV in HeLa cells abolishes functional *p53* and pRb. In this cell line, ectopic expression of *p53* leads to cell death but the lethal effect of *p53* can be reverted by cotransfecting pRb [27]. Additional support for a protective role of pRb against apoptosis comes from the observation that pRb is the target of caspases. pRb is cleaved in different apoptotic systems such as tumor necrosis factor-, staurosporine-, Fas- and cytosine arabinoside-induced apoptosis [41–43]. The cleavage eliminates 42 amino acids at the C-terminal of the protein and it is blocked by caspase tetrapeptide inhibitors [41,42]. The deletion does not affect the growth-suppressive function of the protein but impairs its interaction with Mdm2 [43]. Mdm2, similar to pRb, is a caspase substrate [44]. Mdm2 may function with pRb in inhibiting apoptosis. Its function may be simply to associate with pRB and to maintain pRb protein stability or, in a more complex model, it may cooperate with pRb in directly blocking E2F and *p53* apoptotic pathways.

The role of pRb in apoptosis has yet to be clarified. Studies conducted on E2F-1 may help clarify the anti–apoptotic function of pRb. Transgenic mice null for E2F-1 show inappropriate cell proliferation in the thymus and lymph nodes. Considering E2F-1's role in stimulating cell cycle progression, the expected effect of inactivating E2F-1 is hypoproliferation. The explanation for this surprising result may be that E2F-1, in addition to promoting cell proliferation, controls apoptotic pathways. Indeed, E2F-1 mutants unable to interact with pRb show an even greater ability to induce apoptosis. This suggests that pRb has the ability to regulate E2F-1 function during the cell cycle, but also inhibits the apoptotic pathway that E2F-1 stimulates. In fact, E2F-1 cannot induce apoptosis when pRb is coexpressed [38]. The current data indicate that pRb inhibits apoptosis by avoiding improper activation of E2F-1.

The last argument remaining to be discussed with respect to pRb is the interplay of this molecule with *p53* in regulating cell cycle arrest and apoptosis (Figure 4). As discussed above, in some circumstances, *p53* can induce cell arrest, but in others, it triggers apoptosis. pRb is among the different cellular parameters that leads to one pathway or to the other. To better clarify the cooperation between pRb and *p53*, mice heterozygous for pRb and null for *p53* were generated [45]. In this case, in addition to the tumors that each individual mutation gene causes, other tumors

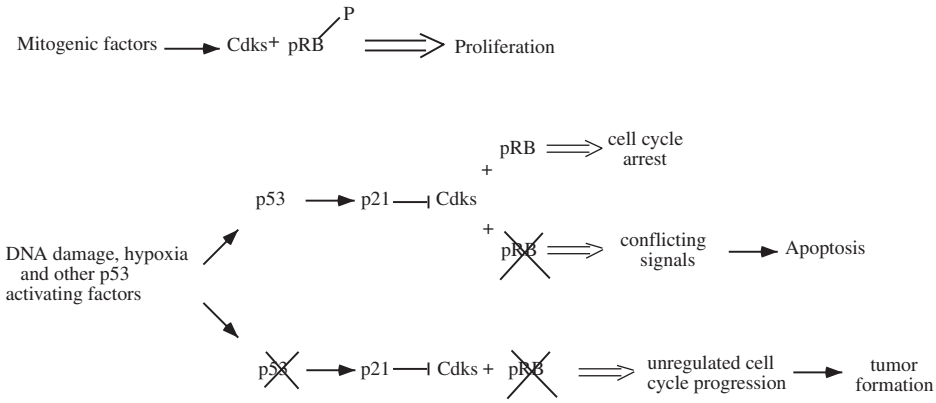

Figure 4. *p53 and pRb cooperation. In normal cells, mitogenic factors and antiproliferative signals regulate cell cycle proliferation and influence the phosphorylation state of pRb. In damaged cells, p53 is activated and causes cell cycle arrest by inducing p21 and by inhibiting pRb phosphorylation by Cdks. If pRb is mutated, the cell cycle is not arrested and the conflict between the p53 signal to stop cell growth and the Cdk signal to proliferate leads to apoptosis. If p53 also is mutated, the cell does not receive a signal to arrest the cell cycle and uncontrolled proliferation leads to tumor formation.*

developed, such as pinealoblastoma and islet tumors. This indicates that *p53* normally represses tumorigenesis when pRb is absent. It does so by inducing apoptosis, as studies conducted with viral proteins have demonstrated. During an infection, the virus must accomplish two goals: 1) to make the cell proliferate abnormally and 2) to shut off apoptotic mechanisms induced by uncontrolled proliferation. Adenovirus SV 40 and HPV solve these problems by simultaneously disrupting *p53* and pRb. E1A, SV40 Tag and HPV E7 bind and inactivate pRb function. SV 40, HPV E6 and adenoviral E1B inactivate *p53*. Studies performed on developing mouse lens have been useful for understanding the effect of viral proteins on pRb and *p53* functions. Expressing E7 in the developing lens causes apoptosis, because the deadly effect of *p53* is not counteracted by pRb. However, if *p53* is eliminated by coexpressing E6, apoptosis is inhibited, resulting in tumor formation [46]. A similar effect is caused by SV40 Tag, which inactivates both *p53* and pRb [47]. SV40 Tag also has been used to examine the effect of pRb and *p53* deficiency in choroid plexus and thymocytes. In these experiments, mutant versions of SV40 Tag, no longer able to bind pRb, cause apoptosis while the wild-type version is able to induce tumorigenesis [48].

Little data are currently available regarding the role of the other two Rb family members, pRb2/p130 and p107, in apoptosis. It is probable that these two proteins have roles in apoptosis because they share other characteristics with the better known pRb. Data supporting a p107 anti–apoptotic role come from a study conducted in mice. The liver and the central nervous system of pRb−/−, p107−/− embryos show more extensive apoptosis than pRb−/− embryos. In addition, the double knock-out animals die 2 days prior to the single mutant [49].

Cdks and Cyclins

Accumulating data show that Cdk/cyclin complexes influence the decision of whether a cell lives or dies. However, it is unclear exactly how Cdks participate in this decision and if their involvement is direct or indirect.

In favor of direct participation of Cdks in apoptosis, there are several studies that illustrate that cyclin A/Cdk complexes positively influence cellular apoptosis. For example, during the effector phase of cell death, cyclin A/Cdk complexes are activated in the nucleus, and their activation depends upon caspases [50]. Another study performed in lymphocytes shows that cyclin A/Cdk2 and cyclin A/Cdc2 activities are necessary during HIV-1 Tat-induced apoptosis [51]. Moreover, dominant negative mutants of Cdc2, Cdk2 and Cdk3 block TNF-induced apoptosis in HeLa cells and can be reverted by coexpressing cyclin A [52]. Additional data for an active role of Cdks in apoptosis come from studies performed on Cdk2 activation in apoptotic thymocytes. In these cells, Cdk2 activation is required for cell death and only occurs subsequent to Bax expression or Bcl-2 repression [53]. These studies support that Cdk activity is modulated by known apoptotic factors working in the induction or execution phases of apoptosis.

However, during apoptosis, Cdk activation can be only a consequence and not the cause. Indeed, the induction of apoptosis does not directly depend on Cdk activity in every case, but on cellular context such as the generation of conflicting signals for proliferation and for cell cycle arrest. In these situations, Cdks may function as signals to sensitize cells to apoptosis. For example, a recent paper by Chen *et al.* [54] demonstrates that a short peptide that impairs the interaction between Cdk2 and its cyclin partner inhibits the activity of Cdk2 and consequently induces apoptosis. However, the inactivation of Cdk complexes also causes a deregulation of E2F activity in transformed cells that are programmed to proliferate. This results in conflicting signals of growth and arrest, which is resolved with the death of the cell.

Cdc2 activity is necessary for cell death in many systems [55]. For example, a dramatic increase in Cdc2 kinase characterizes Granzyme B-induced apoptosis in lymphoma cells and the inactivation of this kinase blocks also the

proapoptotic action of this protease. Cdc2 activity also plays an important role in staurosporine- and in HIV-1 Tat-induced apoptosis. Induction of cyclin B/Cdc2 activity occurs also during apoptosis in taxol-induced apoptosis [56]. However, abolishing Cdc2 function does not impair apoptosis induction in several apoptotic systems such as etoposide, dexamethasone, UV irradiation, serum starvation or Fas-dependent apoptosis [55]. Cdc2 cannot be considered a universal regulator of apoptosis or at least an essential component for all apoptosis. In many cases, in particular when apoptosis is induced in G2 or M-phase, the activation of cdc2 activity could just lead to mitotic catastrophe.

PITSLRE is a Cdk whose role in the cell cycle remains to be established. However, its apoptotic involvement is well demonstrated. Overexpressing PITSLRE induces apoptosis [57] and PITSLRE kinase activity increases during Fas-dependent apoptosis and TNF-mediated apoptosis [58]. Moreover, PITSLRE is cleaved by caspases. The cleavage removes the PITSLRE N-terminus, thereby activating the kinase [57]. During Fas-dependent apoptosis, PITSLRE is phosphorylated and the phosphorylation enhances the N-terminal cleavage [59]. No further information is now available regarding the cellular function of PITSLRE. No cyclins were found to be associated with this kinase. This fact could explain that PITSLRE is a Cdk-related protein involved in events far from the regulation of the cell cycle. It still remains to explain the role of this protein in apoptosis and to find its location in the apoptotic pathway.

Among the cyclins, the involvement of cyclins D in apoptosis has been studied extensively. Cyclin D1 actively promotes apoptosis. Cyclin D1 overexpression causes apoptosis in neuronal differentiated N1E-115 cells and fibroblasts [55]. Apoptosis induced by serum starvation or hydroxyurea is characterized by increased expression of cyclin D1 [60]. Among the cyclin D family of proteins, cyclin D3 also is involved in apoptosis. Janicke *et al.* [61] found that cyclin D3 promotes TNF-induced apoptosis. As for Cdks, upregulation of cyclin D expression results in pushing cells to cycle. Therefore, the apoptotic effect of cyclins may depend on the concomitant signals of arrest, such as differentiation or serum starvation, and proliferation such as cyclin synthesis. Their own synthesis may function to sensitize cells to undergo apoptosis. Therefore, in these situations, the cyclins may not be principle players in these apoptotic pathways but part of an unbalanced mechanism.

Cdk Regulators

Observations supporting the apoptotic role of Cdks come from studies performed on some Cdk regulators.

Among the CKIs, p21 is one of the most highly studied for its involvement in apoptosis. According to several studies, p21 is able generally to rescue cells from programmed cell death during phenomena such as myocytes differentiation [62] and NGF-induced neuronal differentiation [63]. p21 is able to repress DNA-damage-induced such as X-ray-irradiation- or adriamycin-dependent apoptosis [64]. The involvement of Cdk2 in this process is demonstrated by the fact that a p21 mutant unable to bind Cdk2 cannot repress apoptosis.

In contrast to p21, p27's role in apoptosis is less clear. It has been demonstrated that overexpression of p27 can cause apoptotic death in several human cell lines [65,66]. However, p27 may also have anti–apoptotic effects. Hiromura *et al.* [67] showed that $p27-/-$ mesangial cells and fibroblasts are susceptible to serum starvation apoptosis. Restoring p27 presence rescues the cells from cell death. In this case, apoptosis is caused by enhanced cyclin A/Cdk2 activity because of a lack of p27.

Recent data demonstrate that p21 and p27 are targets for caspases, further linking these molecules to apoptotic pathways. During serum starvation-induced apoptosis in endothelial cells, the C-terminus of p21 and p27 is eliminated and their association with Cdk2 is reduced. Dominant negative Cdk2 and a mutant p21 that is no longer cleaved by caspases suppress apoptosis [68]. p21 is a caspase target also during γ-irradiation-induced apoptosis [69] and in TNF-induced apoptosis [70]. p27 is cleaved by caspase-3 to generate a p23 form during G1 arrest in mouse hybridoma and in human myeloma cells. The p23 form is localized to the cytoplasm [71].

p57, another CKI, appears to be involved in apoptosis; its ablation in transgenic heterozygous mice causes apoptosis and delayed differentiation [72].

With regard to p16, it has been observed that adenoviral overexpression of p16 causes G1 arrest and prevents drug-induced apoptosis in glioma cells [73]. In contrast with this observation, it was found that p16 and *p53* coexpression induces cell death in several cell lines [74]. It is likely that the cellular genetic environment plays a critical role in this activity. p16 overexpression is able to induce apoptosis only in *RB*-negative cells such as HeLa cells. In pRb-positive cells, p16 induces cell cycle arrest; the two inhibitors, p18 and p27, have similar effects [75].

The above studies suggest that Cdk inhibitors are indirectly involved in apoptosis at best. Their hyperactivation or mis-regulation leads to improper Cdk activity, which could impart conflicting signals for cell division or arrest. No linkage has been found between these molecules and apoptotic regulators that lead us to suppose a direct influence on the induction or execution of apoptosis.

Conclusion

The regulation of cell proliferation and cell death can share common molecules in multicellular organisms. Indeed, several genes involved in cell cycle regulation are also involved in apoptosis.

Myc's role in cell proliferation and apoptosis has been studied extensively. Evan proposed that Myc directly activates genes involved in proliferation and apoptosis. Mitogens stimulate Myc's proliferation pathway, while anti-apoptotic factors, such as Bcl-2, shut down Myc's apoptotic pathway. When other signals inactivate these anti–apoptotic factors, Myc induces cell death.

Half of all human cancers is characterized by a mutated *p53*. *p53* regulates a cell's fate by inducing either death or cell cycle arrest. The specific pathway chosen depends upon a variety of factors such as the extent of DNA damage and on the genetic background of the cell. In addition, the presence of functional p21 and the cross-talk with pRb are critical determinants in *p53*'s role.

pRb not only is a growth suppressor but also has a protective function against apoptosis. Additional support for a protective role for pRb against apoptosis comes from the observation that pRb is the target of caspases. Current data indicate that pRb inhibits apoptosis by properly regulating the activation of E2F.

Cdks also have been implicated in apoptosis. Several studies demonstrate the active participation of cyclin A/Cdk complexes, Cdc2, cyclin D1, and PISTALRE in apoptosis. Further observations supporting the apoptotic role of Cdks come from studies performed on Cdk regulators. While Cdks do not appear to belong to the apoptotic machinery, they do activate several pathways that converge toward apoptosis. They may function to sensitize cells that are receiving inappropriate growth signals to undergo apoptosis. Cdk involvement in apoptosis is cell-type-specific, however, and also depends on environmental conditions and differentiation states.

In conclusion, the interplay of cell cycle regulators and apoptotic factors influences the destiny of a cell in multicellular organism in order to regulate tissue homeostasis. It becomes a matter of life and death and the survival of the organism lies in the balance. Deregulation of cell proliferation and/or programmed cell death affects the physiology of an organism. Potentially, this could lead to pathological conditions, including cancer and AIDS [2,76]. Understanding the linkage between cell cycle and apoptosis is of primary importance in the search for new therapeutic strategies to combat these diseases.

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