Cell proliferation and apoptosis
Ming Guo* and Bruce A Hay†

Cell proliferation and cell death are essential yet opposing cellular processes. Crosstalk between these processes promotes a balance between proliferation and death, and it limits the growth and survival of cells with oncogenic mutations. New insights into the mechanism by which strong signals to proliferate and activation of cyclin-dependent kinases promote apoptosis have recently been published, and a novel cell cycle regulated caspase inhibitor, Survivin, has been described.

Addresses
*University of California in Los Angeles (UCLA) Medical Center, Department of Neurology, C-128 RRMC, 710 Westwood Plaza, Los Angeles, CA 90095, USA; e-mail: mingfy@itsa.ucl.edu
†Division of Biology, MC 156-99, California Institute of Technology, 1200 East California Boulevard, Pasadena, CA 91125, USA; e-mail: haybruce@its.caltech.edu


Cell proliferation and apoptosis are crucial for normal development and tissue-size homeostasis in the adult. Cancer results when clones of mutated cells proliferate inappropriately, disrupting this balance. One mechanism for maintaining size homeostasis is a requirement for factor-dependent signaling from the environment for cell survival [1]. Cells that exhaust local supplies of these factors or that move to new locations away from the source will die. Although this mechanism of growth control is certainly part of the story, it is not sufficient to limit the expansion of clones that have a proliferation- or survival-promoting mutation. This is because either kind of mutation would be expected to cause an increase in the number of mutant cells relative to their normal neighbors.

A number of observations suggest that signaling between the proliferation and cell death machinery occurs: these include the observation that mutations that promote inappropriate proliferation or that promote proliferation in the face of ectopic bcl-2 expression, dramatically promote cancer development [3]. These observations suggest that an important function of communication between the proliferation and death pathways is to prevent the survival and expansion of clones of aberrant cells. Here we review several recent developments in our understanding of how signals associated with proliferation interface with the cell death machinery. We also discuss a new cell death regulator that IAP (inhibitor of apoptosis) family caspase inhibitor known as Survivin and discuss potential roles it might play.

Oncogene-induced apoptosis
A number of oncogenic mutations drive the cell to proliferate. Examples include mutations that cause overexpression of genes encoding cyclin D1, members of the myc family, adenovirus E1A, the G1 transcription factor E2F-1, as well as loss of function mutations in the G1 checkpoint retinoblastoma gene RB1. However, deregulation of these genes also often results in induction of apoptosis and increased sensitivity to a variety of apoptosis inducing agents [3]. These findings, in conjunction with observations showing that the presence of growth factors can prevent oncogene-induced death and that growth factor removal potentiates such death, have suggested a dual signal model [3,4]. This model proposes that a proliferation stimulus leads to activation of a death signal, either directly as a consequence of entry into the cell cycle or through activation of a parallel pathway, and that successful proliferation can only occur if the apoptotic program is suppressed.

Although it is clear that a strong, oncogenic stimulus to proliferate is associated with death signaling, an important unresolved question is whether normal cell proliferation results in the production of a death stimulus that is qualitatively similar. If so, how is it that cells do not die during normal proliferation? There are several possibilities. Control of entry into the cell cycle by D-type cyclins and their cyclin-dependent kinase (CDK) partners may be one important regulatory mechanism. In normal cells, activation of molecules that drive cells into S phase, such as E2F and c-myc, occurs downstream of cyclin D-CDK-dependent phospho- rylation of Rb family members. The increase in cyclin D levels, which drives this process, occurs as a consequence of growth factor signals from the environment that increase both the synthesis of cyclin D and its assembly with catalytic CDK partners. CDK4 and CDK6 [5]. Signal transduction by these same growth factors also acts to prevent apoptosis in a number of ways [6]. In contrast, when the expression of genes such as those encoding cyclin D itself, c-myc, E2F or adenovirus E1A (which leads to activation of E2F) is uncoupled from the presence of growth factors, cell cycle entry and associated death signaling may occur without concomitant anti-apoptotic signaling, resulting in cell death (Figure 1). It may also be that the levels of survival signaling are not normally limiting for cells undergoing normal proliferation but...
standing of how oncogenic proliferation signals lead to pathways specifically in tumor cells requires an understanding of how oncogenic proliferation signals lead to activation of the death machinery, and the relationship these signals have to any that are produced during normal cell proliferation. One important conceptual advance has come from the finding that, at least in some cases, oncogene expressing cells that are resistant to chemotherapy are able to initiate activation of components of the apoptosis machinery. Thus the machinery can remain activatable, but somehow inhibited. Other recent insights into proliferation-associated death signaling and effector mechanisms are discussed below.

**The p19ARF-p53 pathway**

One of the most important links between the proliferation and cell death machinery is the tumor suppressor p53, which promotes cell cycle arrest or apoptosis in response to DNA damage or a strong oncogenic stimulus to proliferate [7]. Recent evidence shows that oncogene expression and DNA damage use different mechanisms to induce p53-dependent apoptosis [8]. The importance of the oncogene-dependent p53 death pathway is illustrated by the fact that most tumors have disruption of either p53 or an upstream activator of this pathway, the p19ARF tumor suppressor. Expression of oncoproteins such as those encoding E2F-1, c-myc, E1A or oncogenic versions of Ras result in the accumulation of p19ARF [9•,10•,11••,12••], the product of an alternative reading frame (ARF) from the INK4A/ARF locus [13]. This induction occurs through multiple mechanisms [9•,12••], and p19ARF acts in several different ways to promote p53 stabilization and function [14–17]. Oncogene expression in p19ARF−/− cells results in a strongly attenuated induction of p53 (though it is not lost completely) and these cells do not undergo apoptosis [11••,12••]. Also, normal cells that survive senescence crisis or c-myc overexpression generally show mutations in either p53 or p51ARF but not both, consistent with the idea that these genes function in the same death-promoting pathway [12••,18]. Furthermore, p19ARF−/− mice, like those lacking p53, are highly prone to tumor development [18]. Importantly, however, p19ARF−/− cells show a normal induction of p53 following exposure to DNA damaging agents [18]. Also, cells lacking elements of the DNA-damage-dependent p53 pathway are normal with respect to oncogene-dependent p53 induction [19•]. Thus DNA-damage and oncogene expression induce p53 through separate pathways. p53 promotes apoptosis downstream of DNA damage or an oncogenic proliferation stimulus through a number of mechanisms (reviewed in [20]).

**E2F-1: oncogene and tumor suppressor in the retinoblastoma pathway**

Loss of Rb or deregulation of upstream regulators of Rb occurs in essentially all human tumors. One consequence of this deregulation is derepression of E2F family members, resulting in the transcription of genes required for S phase [21]. Loss of function of Rb is thus associated with increased proliferation; however, cell death is also prominent in Rb mutants [22]. Recent evidence demonstrates that E2F-1 plays an important role to limit the
oncogenic consequences of Rb loss by promoting apoptosis through activation of several pathways, including p19ARF-p53 (Figure 2). E2F-1 is a potential oncogene because overexpression can drive entry into S phase and overcome G0 arrest induced by inhibition of G1 CDK activity or irradiation. However, in addition to promoting cell cycle activation, E2F-1, but not other E2F family members, can promote apoptosis through several different mechanisms [21]. These observations suggest that E2F-1 might function as a tumor suppressor, acting to signal cell death downstream of the loss of Rb function. Evidence for such a role comes from observations showing that E2F-1-deficient mice are predisposed to tumor formation [23], and that loss of E2F-1 can suppress apoptosis occurring in cells that have compromised Rb function [24••]. On the basis of these observations one might expect that loss of E2F-1 would promote cell growth. However, evidence that the choroid plexus this is not the case because E2F-1 is also required for effective tumor cell proliferation [24••].

An important question for the future is to determine if E2F-1 is required for the proliferation of other tumor types. Because loss of Rb function occurs in many cancers, therapies aimed at potentiating E2F-1's ability to kill seem appropriate. One approach toward this end utilizes oligopeptides that contain a docking-site motif for cyclin-CDK complexes. Normally, S-phase phosphorylation of E2Fs by cyclin A–CDK2 causes the E2Fs to dissociate from DNA, thus reducing their activity. Peptides with the docking-site motif decrease the ability of cyclin A–CDK2 to phosphorylate substrates, including E2F-1, resulting in increased E2F-1 activity. In cells with compromised Rb function, which already have elevated levels of E2F-1, increasing E2F-1 activity further tips these cells — but not normal cells which have much lower levels of E2F-1 — towards apoptosis [25•]. In other contexts, described below, in which E2F-1 levels are unlikely to be high, blocking CDK activity suppresses apoptosis.

**Linking oncogene activation to the cell death machine**

Most if not all apoptotic cell death signaling pathways ultimately lead to the activation of members of the caspase family of proteases, which act as signal transducers and death effectors [26]. There are two well-described pathways by which caspases that function as signal transducers (known as apical or upstream caspases) become activated. In one pathway initiating at the plasma membrane, ligand binding to a death receptor (CD95 being one example) results in recruitment of procaspase-8 into a multiprotein complex in which caspase autoactivation and transactivation occurs [27]. In a second pathway, cellular stress of various sorts causes the release of mitochondrial cytochrome c. This, in association with a cytoplasmic protein known as Apaf-1, recruits caspase-9 and leads to caspase-9 activation [28,29]. Apical caspases cleave downstream, or executioner caspases. Executioner caspases are thought to have a major role in cleaving cellular substrates that lead to cell death.

Several sets of observations point towards an important role for the cytochrome c, Apaf-1, caspase-9 pathway in mediating oncogene-dependent death signaling. Mouse embryo fibroblasts undergo p53-dependent apoptosis in response to c-MYC overexpression. Death, but not p53 induction, is blocked in versions of these cells in which the genes encoding Apaf-1 or caspase-9 have been knocked out.
It is likely that oncogene expression can act as several different points to regulate activation of this pathway. Thus, biochemical fractionation of cells expressing E1A shows that Apaf-1 and caspase-9 are required for the death of cells expressing adenovirus E1A [31,32••].

Cytoplasmic cytochrome c in these cells does not kill, but rather makes the cell sensitive to death stimuli transduced by growth factor signaling pathways that lead to the activation of the serine/threonine kinase Akt, or by cytoplasmic p21 because similar caspase activating activity is seen in extracts of cells expressing E1A and the adenovirus E1B genes [31], which inhibit a number of apoptotic pathways including those dependent on p53 or bax-mediated release of mitochondrial cytochrome c [33].

Control of the release of cytochrome c defines another major site of death regulation by oncogenes. Thus in Rat-1 cells overexpressing c-MYC, cytochrome c release occurs very early and does not seem to require caspase activation or the activation of other death pathways such as p53 or CD95. Cytoplasmic cytochrome c in these cells does not kill, but rather makes the cell sensitive to death stimuli transduced by growth factor signaling pathways that lead to the activation of the serine/threonine kinase Akt, or by cytoplasmic p21 because similar caspase activating activity is seen in extracts of cells expressing E1A and the adenovirus E1B genes [31], which inhibit a number of apoptotic pathways including those dependent on p53 or bax-mediated release of mitochondrial cytochrome c [33].

CDKs and CDK inhibitors: engines of the cell cycle, ... and cell death? p53-dependent cell cycle arrest occurs as a consequence of induction of the CDK inhibitor p21 [31,32••]. p21 acts at several points — during G1, and at the G1/S phase transition — to block cell cycle progression. It does this by inhibiting cyclin-CDK activity, and by inhibiting DNA replication but not DNA repair as a consequence of interactions with the proliferating cell nuclear antigen [31].

However, p21 also seems to have an anti-apoptotic function downstream of p53 induction because a decrease or loss of p21 gene expression in cells that would normally undergo cell cycle arrest leads instead to apoptosis [38,39]. Thus, the relative levels of p21 and p53-dependent death signals may determine whether cells undergo p53-dependent arrest or death. As described below, caspase cleavage of p21 provides one mechanism by which this balance may be tipped towards death. A number of other observations also point to a role for p21 expression in preventing apoptosis [40,41,42••,43••,44–47], and suggest that p21 may suppress apoptosis in several different ways. Thus p21-dependent cell cycle withdrawal can lead to upregulation of survival signaling molecules such as Akt [43], and cytoplasmic p21 can interact with caspases [47] and the apoptosis signal regulating kinase 1 (ASK1) [44]. In addition, p21 appears to play an important role as an apoptosis inhibitor by virtue of its ability to perform one of the jobs it carries out in promoting cell cycle arrest — inhibiting CDK activity.

Evidence that CDK activity is important for promoting apoptosis comes from a number of observations showing that CDC2 and/or CDC20 — or in the case of neurons CDK4 and CDK6 — are upregulated in a number of cells undergoing apoptosis, that manipulations that decrease this activity prevent death, and that CDK upregulation promotes death [41,42••,43,44–48,52,53•,54,55,56•,57]. However, not all apoptotic deaths require CDK activity [35•,58], and there is no evidence implicating them in the control of cell death in model organisms such as Drosophila or Caenorhabditis elegans. How does CDK activity, which normally promotes cell cycle progression, contribute to apoptosis? The answers are not in, but it seems unlikely that death occurs as a consequence of CDC2-dependent induction of an aberrant cell cycle. This is because events typical of cell cycle progression downstream of CDCK activation such as activation of polo-like kinase-1, entry into S phase or spindle formation do not occur [53•,56•].

How is CDC2/CDK activity upregulated? A number of observations, with some exceptions [57], argue that CDK activation occurs downstream of death signal initiation and as a consequence of degradation or caspase-mediated cleavage of negative regulators of CDKs. Thus, the CDK inhibitors p21 or p27 [58] has been observed in a number of situations in which apoptosis occurs [43••,59,60], and overexpression of normal or uncleavable versions of these proteins provides strong protection against death [43••,60]. Degradation of p27 [58] has been observed in other cells [57•]. Finally, cleavage of the CDK negative regulatory kinase weel, which might be expected to compromise its ability to inhibit CDK activity through phosphorylation, has also been documented [56•].

There are several ways in which CDK activation downstream of caspase activation might contribute to caspase-dependent cell death. CDK phosphorylation might function as part of a positive-feedback loop that leads to further caspase activation by decreasing the activity of caspase inhibitors, such as the IAPs, or increasing the activity of other pro-apoptotic molecules. Alternatively, ectopic CDK activity might also lead to induction of other death pathways. The important point is that initiation of a...
caspase cascade, particularly in the presence of caspase inhibitors, might not always be sufficient to sustain an apoptotic response. Activation of CDKs could provide a way of maintaining or amplifying this activity. Such amplification might be required for apoptosis in some contexts but not others, explaining why a requirement for CDK activation during apoptosis is not universal.

Presumably, the key to understanding the roles CDK activation plays in apoptosis will come from identifying the substrates phosphorylated. One possibility is that these substrates are proteins that are involved in similar ways in apoptotic and mitotic cells. These might include proteins contributing to the morphological similarities between apoptotic and mitotic cells, such as cell rounding, nuclear membrane breakdown or chromatin condensation. Alternatively, CDK activation during apoptosis might result in phosphorylation of death-specific targets. If this is correct an important question becomes how CDK activity is targeted to these substrates only during apoptosis? One way to achieve this might involve association of the kinase with novel cyclin partners. Consistent with this possibility, CDK2 activation in apoptosing thymocytes does not appear to be associated with its normal partners, cyclins A or E, and CDK2 activation requires protein synthesis [52*].

However, the identity of the postulated cyclin partners is unknown, and in other situations apoptosis-associated CDK or CDK activity does occur in association with normal cyclin partners [49*].

**Survivin the cell cycle?**

Some oncogene-expressing cells can survive the mitochondrial dysfunction associated with cytochrome c release if subsequent caspase activation is blocked [38*]. Although the possibility that these cells have acquired second site mutations that prevent cytochrome c release has not been ruled out, these results suggest that a mechanism by which transformed cells could escape death would involve upregulation of caspase inhibitors. Three mammalian IAPs, XIAP, cIAP1 and cIAP2 are expressed broadly in development and in the adult. Several of these are induced by Rel or NF-κB [61] and Survivin is one of a number of contexts [62]. However, overexpression of these IAPs in actual tumors has not been documented. A recent study identified human protein, Survivin [63] (TIAP in the mouse) [46*]. Survivin shows prominent regulation at the transcriptional level. It is abundant during development in proliferating tissues and in tissues in which apoptosis is prominent, but is low or absent in terminally differentiated adult tissues [63,64*]. Most importantly, Survivin is upregulated in a number of common cancers and transformed cell lines [63,63,66*]. Additional evidence suggesting that Survivin may play an important anti-apoptotic role in cell proliferation and cancer progression comes from the findings that Survivin is upregulated in G2/M [64*] and that it is associated with spindle microtubules and seems to require this association for anti-apoptotic activity at least with respect to the apoptosis inducer taxol [67•]. Also, overexpression of the gene encoding Survivin blocks cell death in response to a number of different stimuli [63,64*] and Survivin binds, although not well, to processed forms of caspases-3 and -7 [64*]. Finally, antisense Survivin promotes caspase activation and cell death, at least in HeLa cells [68].

One possibility suggested by these observations is that caspase activity occurs during each cell cycle and that Survivin functions to block this activity. This caspase activity might be required for some aspect of cell division. Alternatively, caspase activity and Survivin could act together as part of a G2/M checkpoint. In such a model Survivin associated with microtubules might be required to block caspase activity during G2/M. Disruption of microtubules would then lead to loss of Survivin function and an increase in death-promoting caspase activity. Survivin’s functions might, however, be more complex than simple caspase inhibition. The worm *C. elegans* and the yeasts *S. cerevisiae* and *S. pombe* also have genes that encode small BIR-containing proteins (BIRPs), which have significant homology to Survivin, and these proteins are required for cytokinesis [69,70].

There are a number of unanswered questions. Is caspase activation in fact a normal part of movement through the cell cycle? Regardless of whether it is or not, what is the source of caspase activity found in cells with decreased Survivin function? Does it arise as a consequence of the loss of Survivin or as a secondary consequence of loss of a Survivin-dependent function that leads to caspase activation? Analysis of Survivin knockouts should be very illuminating. In particular it will be important to determine if cells that lack Survivin are able to progress through the cell cycle if caspase activity is blocked using other caspase inhibitors. If Survivin function as a caspase inhibitor does in fact serve as a downstream roadblock on the way to tumor cell apoptosis, the fact that it is essentially absent in normal postmitotic tissues in the adult makes it an exciting potential therapeutic target. Identifying molecules that can disrupt IAP–caspase interactions then provides one route to inducing tumor cell death [71•].

**Conclusions**

Tissue size homeostasis requires a balance between proliferation and cell death. An oncogene stimulus to proliferation has the potential to disrupt this balance; however, this potential is kept in check because a strong proliferation stimulus also leads to the production of death signals that make these cells more sensitive to environmental conditions such as growth-factor deprivation or hypoxia. The E2F-1–p19ARF–p53 pathway constitutes a vital mechanism by which oncogene-induced death signals are transduced, but other E2F-1, ARF-1, and p53-independent pathways exist as well. Activation of caspase-9 downstream of mitochondrial release...
of cyctronchrome c is an important pathway by which oncogene-dependent death is carried out but the ways in which this pathway becomes activated are still largely unknown. Further downstream, caspase-dependent and independent activation of CDKs contributes to apoptosis in many cell types but the functions and relationship of this activity to that required for cell cycle progression are unknown. Does normal cell proliferation result in the production of a death stimulus that is qualitatively similar to that induced by oncogene expression? The identification of Survivin as a cell cycle-regulated caspase inhibitor also upregulated in tumors suggests that caspase activity may occur normally during phases of the cell cycle. But whether this putative caspase activity functions as a part of a proliferation checkpoint or has other roles is unknown, as are the normal roles of Survivin.

Note added in proof

Two recent reports [72••,73••] demonstrate that an important mechanism by which c-myc induces proliferation involves upregulation of cyclins D1 and D2. Cyclin D-CDK complexes sequester the CDK inhibitors p27 and p21KIP, thus promoting cyclin E-CDK activity. Interestingly, although cyclin D1 and D2 are required for myc-dependent proliferation, they are not required for myc-dependent apoptosis. Thus, these papers show that myc-dependent proliferation and apoptosis signaling are generically separable. Cells from cyclin D2-/- mice that overexpress c-myc should provide a powerful tool for identifying myc's apoptotic targets.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:
• of special interest
•• of outstanding interest


31. Hueber AO, Zornig M, Lyon D, Suda T, Nagata S, Evan GI: Mouse embryo fibroblasts undergo p53-dependent death in response to c-myc overexpression. This paper shows that p53-dependent death, but not p53 expression, is suppressed in cells lacking Apaf-1 or caspase-9. Impotently, the surviving cells are able to proliferate in vitro and form tumors in vivo. This argues that the mitochondrial dysfunction leading to the release of cytochrome c is not so great as to preclude cell growth if caspase activation is suppressed. On the basis of these results one might expect to identity Apaf-1 and caspase-9 as tumor suppressors and caspase inhibitors as dominant oncogenes.


This paper shows that E1A-dependent apoptosis requires Apaf-1 and caspase-9. This paper, in conjunction with earlier work (17), shows that cells expressing E1A are somehow primed to activate caspase-9. The nature of the priming stimulus is unknown. This paper also shows that cells expressing E1A are released to cytochrome c in response to other death stimuli. It is not known if this cytochrome c release occurs as a part of a feedback pathway from active caspase-9 to mitochondria, or if E1A sensitizes mitochondria to cytochrome c release in other ways.


This paper shows that the expression of c-Myc results in an early release of cytochrome c that, although not toxic itself, sensitizes Rat-1 cells to other apoptosis-inducing stimuli. The c-Myc-dependent release of cytochrome c is inhibited by IAP-1, which probably acts through the kinase Akt. These observations suggest a model in which the fate of cells expressing c-Myc depends on control of cytochrome c release and the extent of activation of other death pathways.


This paper shows that E1A-dependent apoptosis requires Apaf-1 and caspase-9. This paper, in conjunction with earlier work (17), shows that cells expressing E1A are somehow primed to activate caspase-9. The nature of the priming stimulus is unknown. This paper also shows that cells expressing E1A are released to cytochrome c in response to other death stimuli. It is not known if this cytochrome c release occurs as a part of a feedback pathway from active caspase-9 to mitochondria, or if E1A sensitizes mitochondria to cytochrome c release in other ways.


This paper shows that the expression of c-Myc results in an early release of cytochrome c that, although not toxic itself, sensitizes Rat-1 cells to other apoptosis-inducing stimuli. The c-Myc-dependent release of cytochrome c is inhibited by IAP-1, which probably acts through the kinase Akt. These observations suggest a model in which the fate of cells expressing c-Myc depends on control of cytochrome c release and the extent of activation of other death pathways.

Despite CDKs and CDK2 activation, other events associated with cell cycle progression do not occur.


