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Cell cycle and apoptosis

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Abstract. Apoptosis and proliferation are intimately coupled. Some cell cycle regulators can influence both cell division and programmed cell death. The linkage of cell cycle and apoptosis has been recognized for c-Myc, p53, pRb, Ras, PKA, PKC, Bcl-2, NF-κB, CDK, cyclins and CKI. This review summarizes the different functions of the proteins presently known to control both apoptosis and cell cycle progression. These proteins can influence apoptosis or proliferation but different variables, including cell type, cellular environment and genetic background, make it difficult to predict the outcome of cell proliferation, cell cycle arrest or cell death. These important decisions of cell proliferation or cell death are likely to be controlled by more than one signal and are necessary to ensure a proper cellular response.

INTRODUCTION

Tissue homeostasis is dependent on the perfect balance between cell proliferation and cell death. The balance between positive and negative signals determines the decision between life or death. An imbalance can result in diseases linked with unwanted apoptosis or unwanted cell growth. A direct link between cell cycle and apoptosis may be supposed from the fact that a number of similar morphological features exist between mitosis and apoptosis. These include substrate detachment, cell rounding, cell shrinkage and chromatin condensation. (Of course important and determining differences exist, e.g. at the level of DNA, which is fragmented in apoptotic cells while it is segregated during mitosis.) Different components common to apoptosis and the cell cycle have been identified and provide a second rationale for linking cell cycle and apoptosis. Our primary focus here will be on the dual role of some proteins from cell cycle and apoptotic pathways, including c-Myc, p53, pRb, Ras, protein kinase A (PKA), protein kinase C (PKC), Bcl-2, NF-κB, CDK, cyclins and CKI. Following stimulation, these proteins may induce cell proliferation, cell cycle arrest or cell death; the different outcome depends on different variables. The genetic background of the cell is important as is the cellular micro-environment. Also, the extent of DNA damage and the level of different proteins contribute to the life or death decision-making process.

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Detailed discussion of the proteins is beyond the scope of this review and because detailed reviews are available,(Sionov & Haupt 1999; Adjei 2001; Oster *et al.* 2002) only some major aspects of their dual function will be described here.

c-MYC

c-Myc is a nuclear phosphoprotein that functions as a transcription factor stimulating both cell cycle progression and apoptosis (Facchini & Penn 1998; Penn *et al.* 1990). Despite the fact that c-Myc has been widely studied since its discovery, many questions about the function and regulation of c-Myc still exist. c-Myc expression is regulated post-translationally through protein phosphorylation and through interaction with other cellular proteins, primary Max (Alvarez *et al.* 1991; Blackwood & Eisenman 1991; Lutterbach & Hann 1994).

c-Myc has a critical role in normal cell cycle progression, especially during transition from G_0 to S phase (Spencer & Groudine 1991). c-Myc is an early response gene, i.e. it responds directly to mitogenic signals to push cells in the G_1 phase of the cell cycle (Fig. 1) (Heikkila *et al.* 1987; de Alboran *et al.* 2001). c-Myc expression is maintained throughout the cell cycle and some observations also suggest a role for c-Myc in G_2 (Hann *et al.* 1985; Mateyak *et al.* 1997). c-Myc can exert its effect on cell cycle progression by the transcription of genes with an important role in cell cycle control, i.e. C*dc25A*, *cyclin D1*, *cyclin D2*, *cyclin E*, *cyclin A*, *CDK1*, *CDK2*, *CDK4* and *E2F* (Born *et al.* 1994; Kim *et al.* 1994; Beier *et al.* 2000). Another important mechanism of the ability of c-Myc to promote cell growth is suppression of transcription of growth arrest and the DNA damage inducible gene 45 (*Gadd45*), *Gadd153* and of the CKI genes *p15*, *p21*, and *p27* (Fig. 1) (Dang 1999; Grandori *et al.* 2000).

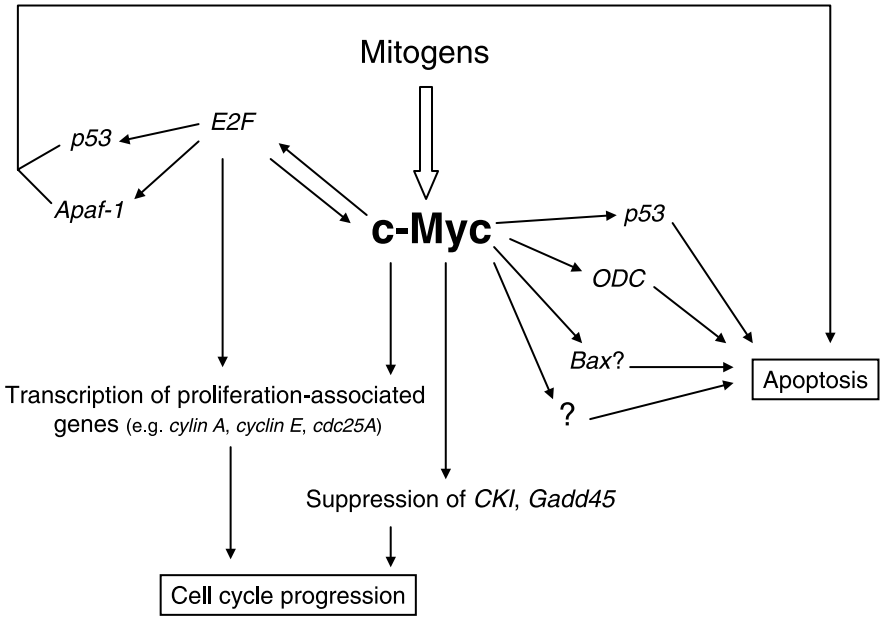

Figure 1. The role of c-Myc in cell cycle and apoptosis.

Besides its role in cell cycle, c-Myc also plays a key role in regulating apoptosis. This role was confirmed in different cell types under a wide variety of physiological conditions and both under- and overexpression of c-Myc can lead to cell death (reviewed in Conzen *et al.* 2000 and Thompson 1998). Until now, the molecular events responsible for c-Myc-induced apoptosis are not well understood. Several components important for cell cycle progression, including cyclin A and Cdc25A, have been implicated in apoptotic systems associated with elevated c-Myc. Cyclin A was elevated in rat-1 A fibroblasts overexpressing c-Myc and undergoing apoptosis (Hoang *et al.* 1994). Cdc25A is a well-established transcriptional target of c-Myc and can induce apoptosis in serum-deprived fibroblasts, as does c-Myc (Galaktionov *et al.* 1996). However, not all the Cdc25A substrates are yet known and its relationship with molecules participating in apoptosis remains to be elucidated (Zornig & Evan 1996; Thompson 1998). Another target for transcriptional stimulation by c-Myc is ornithine decarboxylase (*ODC*) which can cause apoptosis when overexpressed (Packham & Cleveland 1994; Packham & Cleveland 1995). c-Myc-induced apoptosis may involve p53-dependent and -independent pathways. c-Myc transactivates the *p53* gene promotor and increases the half life of p53 (Reisman *et al.* 1993; Hermeking & Eick 1994). However, there does not seem to be a universal requirement for p53 in c-Myc-mediated apoptosis (Hsu *et al.* 1995; Sakamuro *et al.* 1995). c-Myc-induced apoptosis has been shown to correlate with Fas ligand and Fas receptor expression (Wang *et al.* 1998). c-Myc-induced apoptosis also seems to be inhibited by Bcl-2 and Mcl-1 (Bissonnette *et al.* 1992; Fanidi *et al.* 1992; Reynolds *et al.* 1994). c-Myc-induced cytochrome c release involves functionally active Bax and preliminary evidence suggests that c-Myc regulates the transcription of this pro-apoptotic molecule (Duelli & Lazebnik 2000; Mitchell *et al.* 2000; Soucie *et al.* 2001).

A ‘dual signal’ model has been postulated were the ability of c-Myc to drive apoptosis is distinct from its ability to drive cell division (Pucci *et al.* 2000; Oster *et al.* 2002). This model is supported by the observation that different subregions of the c-Myc N-terminal domain can control distinct biological functions, including apoptosis (Chang *et al.* 2000; Conzen *et al.* 2000; Nesbit *et al.* 2000). However, this issue cannot be directly addressed until c-Myc target genes essential for apoptosis have been clearly identified. The factors that determine the decision of inducing either cell division or cell death needs to be further addressed. In addition, c-Myc expression is tightly linked to the extracellular milieu and the function of c-Myc is also probably influenced by the extracellular environment (Oster *et al.* 2002).

p53, pRB AND E2F

The regulation and the role of the tumour suppressor proteins p53 and pRb and of the transcription factor E2F have been discussed in the previous review. p53 is widely recognized as a protein functioning during the cell cycle (i.e. an inducer of cell cycle arrest) and apoptosis (Levine 1997; Sionov & Haupt 1999). p53 regulates these processes by transactivating genes involved in different cellular functions, but p53 also activates transcription-independent mechanisms of apoptosis (Haupt *et al.* 1995; Agarwal *et al.* 1998). pRb inhibits cell cycle progression by interacting with transcription factors such as E2F; when pRb becomes phosphorylated, E2F is released and stimulates proliferation. Besides cell cycle inhibition through E2F suppression, pRb has also been shown to suppress apoptosis. For instance, Rb-deficient embryos show defects in fetal liver haematopoiesis, neurogenesis and lens development, and extensive apoptosis was observed in these tissues (Morgenbesser *et al.* 1994; Macleod *et al.* 1996). The mechanisms by which pRb/E2F influences apoptosis remain unknown. E2F has been shown to induce the expression of the

pro-apoptotic factor *Apaf-1* and evidence suggests a role for E2F in apoptosis following DNA damage (Blattner *et al.* 1999; Moroni *et al.* 2001). E2F cannot induce apoptosis when pRb is co-expressed and pRb possibly has an anti-apoptotic effect through the inhibition of E2F (Fan *et al.* 1996; Pucci *et al.* 2000).

p53 and pRb/E2F may be directly linked in cell proliferation and apoptosis. Activated p53 causes a G_1 arrest by inducing p21, followed by an inhibition of cyclin/CDK. In these conditions, pRB is not phosphorylated and cells do not progress through the cell cycle. In contrast, free E2F directly induces *p53* transcription, thus connecting the pRb/E2F pathway to p53-dependent apoptosis (Hiebert *et al.* 1995). Each of both tumour suppressors (p53 and pRb) may thus be able to compensate for the loss of the other (King & Cidlowski 1998).

RAS

Ras is a membrane-localized G protein. By activating the mitogen-activated protein kinase (MAPK) signalling cascade and the phosphatidyl inositol 3-kinase (PI3K) pathway, it has a role in cell proliferation as well as in inhibition and promotion of apoptosis (Adjei 2001). Upon activation by the MAPK kinase kinase (MAPKKK) Raf and the MAPK kinase (MAPKK) MEK (MAP/ERK kinase), the MAPK ERK (extracellular signal-regulated kinase) translocates to the nucleus, where it phosphorylates transcription factors, resulting in expression of genes involved in cell cycle progression (e.g. *cyclin D1*) (Fig. 2) (Avruch *et al.* 1994; Hagemann & Rapp 1999). By phosphorylating and inactivating Bad, activated Raf, MEK and ERK contribute to the

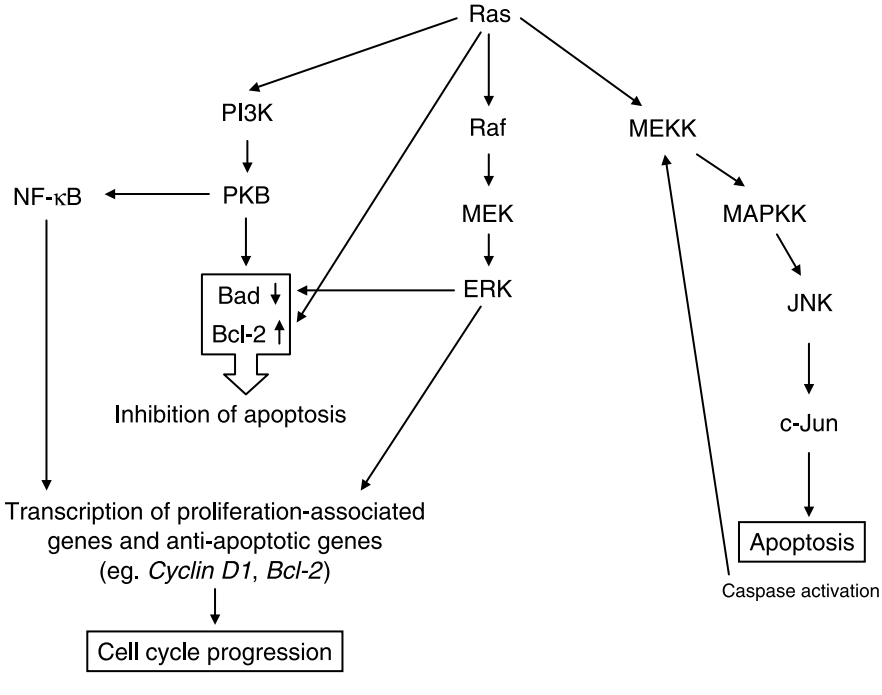

Figure 2. The role of Ras in cell cycle and apoptosis.

inhibition of apoptosis. Activation of PI3K by Ras also inhibits apoptosis by activating protein kinase B (PKB) (also known as Akt), which also results in phosphorylation and inactivation of Bad (Kauffmann-Zeh *et al.* 1997). PKB also phosphorylates and inactivates caspase-9 (Cardone *et al.* 1998). However, PKB can also affect cell survival by exerting its effect on the transcription factor NF-κB via phosphorylation of the NF-κB regulator IκB (Romashkova & Makarov 1999). The anti-apoptotic effects of Ras can also be mediated by up-regulation of Bcl-2 and other anti-apoptotic members of the Bcl-2 family (Kinoshita *et al.* 1995).

Ras-mediated apoptosis is promoted by the c-Jun N-terminal protein kinase (JNK) pathway and can be brought about through the death receptor pathway or by cellular stress e.g. ultraviolet light or osmotic shock (Franklin & McCubrey 2000). In the JNK pathway, Ras activates the MAPKKK MEKK which activates different MAPKK to phosphorylate the MAPK JNK (Fig. 2). Substantial evidence has implicated JNK as an essential component of the apoptotic cascade (Tournier *et al.* 2000). All the elements are still not clearly defined, but active JNK phosphorylates the transcription factor c-Jun, which is crucial for the induction of apoptosis (Leppa & Bohmann 1999). JNK activation following growth factor withdrawal results in the up-regulation of FasL expression and apoptosis (Le Niculescu *et al.* 1999). MEKK can be cleaved and activated by caspases which results in JNK activation and potentiation of apoptosis (Cardone *et al.* 1997; Widmann *et al.* 1997). Under certain circumstances, JNK signalling can also promote cell survival; for instance activated c-Jun has been suggested to have a protective role in DNA-damage induced apoptosis in human tumour cells (Potapova *et al.* 2001). However, the underlying mechanisms for this effect is not yet understood (Leppa & Bohmann 1999).

PKA

PKA is a serine/threonine kinase that is activated by cyclic adenosine monophosphate (cAMP). Increased cAMP can both inhibit and promote apoptosis (Franklin & McCubrey 2000). cAMP-induced apoptosis is mediated by inhibition of the Ras/Raf/MEK pathway (Hafner *et al.* 1994; Marshall 1995). In B cells, activation of PKA caused a reduced expression of the anti-apoptotic protein Mcl-1, associated with apoptosis (Myklebust *et al.* 1999). In contrast, PKA activation can result in the phosphorylation of Bad – at the same site as that induced by Raf and MEK – and is associated with the anti-apoptotic effects of PKA (Harada *et al.* 1999). Effects of PKA on apoptosis are likely to be largely dependent on the cell type and the mechanisms by which apoptosis is induced (Franklin & McCubrey 2000).

BCL-2

As discussed earlier, Bcl-2 mainly has an anti-apoptotic function, but this function can be lost by multi-site phosphorylation (Haldar *et al.* 1995). The regulation of the function of Bcl-2 mainly involves interactions with other proteins of the Bcl-2 protein family, but phosphorylation may also be a crucial event in the regulation of its function (Haldar *et al.* 1995; Yamamoto *et al.* 1999). Several signal transduction pathways can be involved in Bcl-2 phosphorylation. Bcl-2 is phosphorylated on serine/threonine residues and the Bcl-2 kinase(s) is/are serine/threonine kinase(s). CDK1 is a candidate Bcl-2 kinase and has been shown to phosphorylate Bcl-2 (Furukawa *et al.* 2000). However, other studies demonstrated that CDK1 did not phosphorylate

Bcl-2 (Scatena *et al.* 1998; Yamamoto *et al.* 1999). JNK was repeatedly indicated as a potential Bcl-2 kinase (Blagosklonny 2001). It has been reported to phosphorylate Bcl-2 at four serine/threonine sites (Maundrell *et al.* 1997). However, it has been suggested that several kinases may be involved in the phosphorylation of Bcl-2 (Blagosklonny 2001).

Importantly, very high levels of Bcl-2 can promote cell death (Shinoura *et al.* 1999). Bcl-2 can also modulate the cell cycle in a way that is different from the inhibitory effect on apoptosis (Linette *et al.* 1996). Bcl-2 gene expression can result in an increase of 30–60% in the length of G_1 phase and under suboptimal conditions, Bcl-2 promotes exit into quiescence and retards re-entry into the cell cycle (Mazel *et al.* 1996; Adams & Cory 1998).

NF-κB

The transcription factor NF-κB up-regulates several survival factors, however, it has also been associated with anti-apoptotic activities (Baichwal & Baeuerle 1997; Hinz *et al.* 1999). NF-κB up-regulates the expression of the anti-apoptotic Bcl-2 family members, Bcl-2, Bcl-Xl and Bfl-1/A1 (Glasgow *et al.* 2001). Anti-apoptotic activity has also been shown in conjunction with certain apoptotic stimuli, e.g. TNF-α, ionizing radiation and daunorubicin (Beg & Baltimore 1996; Wang *et al.* 1996). Alternatively, there is also evidence for apoptosis-promoting functions of NF-κB (Kaltschmidt *et al.* 2000). For instance, in response to anti-cancer drugs, NF-κB directly transactivates *FasL*, whose gene product contributes to cell death (Kuhnel *et al.* 2000).

CELL CYCLE REGULATORS

Different cell cycle regulators including Wee1, cdc27, p21, p27, pRb and CDK1 are targets for cleavage by caspases. In this way, cell cycle progression during apoptosis is stopped (Jacotot *et al.* 2000). Further cleavage of essential proteins will result in cell death, independently of the cell cycle. Several typical cell cycle regulators like CDK, cyclins and CKI affect apoptotic signalling, however, no clear-cut pro- and anti-apoptotic effects can be described. Little is known about the interaction of cell cycle regulatory proteins with c-Myc and with apoptotic regulators like Bcl-2. Neither is it known whether or how the cell cycle regulators influence mitochondrial pore opening. It will be important to answer these questions, in order to establish the link between cell cycle control and apoptosis.

CDK and cyclins

The role of CDK and cyclins in cell proliferation is widely known and was extensively discussed in the previous review. Contradictory results exist about the role of CDK in apoptosis. Some studies reported a pro-apoptotic activity for CDK; they showed requirement of activated CDK during apoptosis of thymocytes (Gil-Gomez *et al.* 1998; Hakem *et al.* 1999). Some apoptosis-inducing agents (staurosporine, caffeine) can cause induction of CDK1 and CDK2 activity prior to cell death (Meikrantz *et al.* 1994). Dominant negative mutants of CDK1, CDK2 and CDK3 suppress apoptosis, induced by staurosporine and TNF-α (Meikrantz & Schlegel 1996). CDK seems to be required for neuronal cell death (Rubin *et al.* 1994). Inhibition of CDK by flavopiridol and olomoucine protected post-mitotic non-dividing PC12 neuronal cells from apoptotic cell death (Park *et al.* 1996). Inhibition of CDK2 has also been shown to protect thymocytes from

apoptosis, mitochondrial changes and caspase activation (Hakem *et al.* 1999). Our own data, in addition to those of others, show induction of apoptosis in haematopoietic cells in association with CDK inhibition (Arguello *et al.* 1998; Byrd *et al.* 1998; Vermeulen *et al.* 2002a,b). Cyclin D overexpression is associated with apoptosis, although this may depend on concomitant signals of cell cycle arrest (e.g. serum starvation) and cell proliferation (e.g. cyclin synthesis). Taken together, induction of apoptosis depends on the cellular context: conflicting signals for cell proliferation and cell cycle arrest may result in cell death (Kasten & Giordano 1998).

CKI

CDK inhibitors have been suggested to be indirectly involved in apoptosis through regulation of CDK. Improper regulation of CDK can send conflicting signals for cell division and cell cycle arrest. p21 is synthesized during the p53-dependent G_1 cell cycle arrest, where it can have anti or pro-apoptotic properties. For example, overexpression of p21 inhibits radiation-induced apoptosis in human colorectal carcinoma cells, while an inducible expression of p21 sensitizes EJ tumour cells to mitomycin C-induced apoptosis (Lu *et al.* 1998; Fang *et al.* 1999). p27 may also have both pro- and anti-apoptotic effects (Wang *et al.* 1997; Hiromura *et al.* 1999; Lloyd *et al.* 1999).

CONCLUSION

Several genes are common to cell cycle regulation and to apoptosis. The fate of cells is likely to be determined by their interplay. When cells are subjected to adverse (growth) conditions, complex signal transduction networks are initiated. The information received is processed and sent to subcellular organelles. For example, p53 is one protein that plays a key role in the decision to either arrest the cell cycle followed by DNA repair, or to commit cell death. The specific pathway chosen depends upon a variety of factors such as the extent of DNA damage, the presence of functional p21 and its cross-talk with pRb and the genetic background of the cell. The important decisions of cell death or cell proliferation are likely to be controlled by more than just one signal; most likely this is a mechanism that ensures a proper cellular response.

The current understanding of the functions of cell cycle regulators in apoptosis has progressed considerably. However, many questions remain to be answered.

One can imagine catastrophic consequences for the cell, when key players in cell cycle regulation and/or apoptosis are not co-ordinated. The knowledge of the links between cell cycle and apoptosis should be of help in understanding pathological conditions, in addition to identifying new therapeutic strategies.

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