

**CONTROL NODES LINKING THE REGULATORY NETWORKS OF THE
CELL CYCLE AND APOPTOSIS**

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SUMMARY

Depending on the nature of extracellular stimuli and the ensuing intracellular signal transduction pathways, certain transcription factors are activated and subsequently determine the extent of expression of genes involved in cell proliferation, survival and death. These factors are referred to as transcriptional control nodes because they permit the coordination of cell cycle progression and the apoptosis program. This coordination is made possible by the existence of feedback loops in the regulatory networks of the entire system. A review of these networks for the following transcription factors is provided in this chapter: E2F, Myc, p53, and NF- κ B.

Keywords:

E2F, Myc, p53, NF- κ B, cell cycle, apoptosis, regulatory networks, control nodes, modules

1. INTRODUCTION

It is not surprising that the molecular regulatory networks of the cell division cycle and apoptosis are tightly intertwined (1) because when the so-called cell cycle engine ‘overheats’ in certain cells in an organism, a mechanism has to exist to get rid of these cells in order to steer a normal course of development or maintenance of the organism. Thus, it is quite reasonable to expect that at least one pathway leading to the initiation of the cell suicide program is linked to at least one pathway leading to the initiation of the cell cycle. Without such link the coordination between the cell cycle and apoptosis machineries cannot be controlled. In this chapter, it is postulated that the key links are provided by certain transcription factors (TFs) controlling the expression of genes involved in the cell cycle and apoptosis. Activation of these TFs is initiated by extracellular signals transduced by intracellular pathways that directly cause the translocation of the TFs to the nucleus, binding to gene promoters and initiation of transcription. These transcriptional nodes are referred to in this chapter as ‘control nodes’ because they permit the possibility of coordinating the cell cycle and apoptosis. The discussion is organized according to the general classification of signaling pathways and control nodes shown in Figure 1 (see also Ref. 2). The pathways in this figure should be interpreted in a very general way; for example, type I signaling pathways activate type I control nodes which potentiate pathways promoting the cell cycle and apoptosis (‘potentiate’ here means that the pathway does not necessarily trigger activation). Note that the signaling pathways shown in Figure 1 are only those that activate the control nodes but, in general, the discussion will also include both activating and inhibiting signaling pathways and their interactions. Furthermore, an important observation is that

no case as shown in Figure 1 would give rise to a mechanism in which the status of the cell cycle module influences the status of the apoptosis module. Indeed, one can see that there should be at least a pathway from the cell cycle module which feeds back to the transcriptional node or to any upstream signalling pathway that regulates the TF node activity; this feedback loop would generate the situation in which the TF control node activity is a function of the cell cycle module status (i.e. the activity of some cell cycle factor) and therefore the status of the apoptosis module would be an indirect function of the cell cycle module status. Of course, the cell cycle module can directly communicate to the apoptosis module by either regulating the pathways from the TF node to apoptosis and/or the apoptosis module itself.

< **Figure 1 here** >

The known molecular regulatory networks of the cell cycle and apoptosis are quite complex and can overlap. However, as suggested in Figure 1, it will be assumed that these networks are separable and can be modularized by focusing on the primary enzymes that trigger these cellular processes, namely, the cyclin-dependent kinases (CDKs) for the cell cycle and the caspases for apoptosis. A discussion of the basic components and interactions in these modules will be provided in the next section. As will be shown, these modules represent tightly regulated interactions that communicate with the transcriptional nodes in both directions.

The control nodes discussed in depth in this chapter include the E2F, Myc, p53, and NF- κ B transcription factors. E2F and Myc are combined into one node which will

be referred to as the E2F-Myc node; E2F here specifically represents E2F-1 (a member of the E2F family) and Myc refers to c-Myc. These TFs are grouped together because of their synergistic interactions and the fact that they possess many common transcriptional targets. The E2F-Myc node represents Case I in Figure 1. The tumor suppressor function of the protein p53 is mainly attributed to the pro-apoptotic gene targets; in addition, p53 induces the expression of several genes whose protein products inhibit cell cycle progression. Thus, p53 is an example of Case II in Figure 1. Case III is exemplified by the NF- κ B family of dimeric transcription factors which is primarily known for its role in the immune response, but is now increasingly linked to carcinogenesis through pathways that connect to the cell cycle and apoptosis. (Case IV in Figure 1 is not of interest in this chapter). Although there are more transcriptional nodes that can control the cell cycle and apoptosis programs, the above examples are deemed sufficient to illustrate some of the principles needed to understand the operation of transcriptional control nodes as they affect the cell cycle and apoptosis modules

A detailed analysis of the E2F-Myc control node and its interactions with the signaling, cell cycle, apoptosis modules illustrate some important concepts governing the operation of these networks. There are several coupled positive and negative feedback loops present in the regulatory networks, and these loops operate both at the level of a single module and at the level of module-module interactions. Positive feedback loops could lead to switching and bistable behavior while negative feedback loops can generate sustained oscillations. The structures of the regulatory networks of the other transcriptional nodes in terms of feedback loops are therefore discussed to demonstrate how to predict sources of switching and other unstable behaviors. The intrinsic

nonlinearities of these control networks make them very difficult to understand intuitively. Indeed, mathematical modeling and computer simulations are tools that would greatly aid in understanding these complex networks.

2. CELL CYCLE AND APOPTOSIS MODULES

2.1 G1/S Module

Based on a kinetic analysis of the then prevailing consensus G1-S regulatory network of the mammalian cell cycle, Aguda and Tang (3) proposed that the G1 checkpoint called the ‘Restriction Point’ is governed by the activation of cyclin E/CDK2 whose critical regulators include the phosphatase Cdc25A, and a CDK inhibitor (p27Kip1). This picture has not changed much as can be seen in a recent review of the field (for example, see Ref. 4). However, recent experiments with mice knocked out of G1 cyclins and G1 CDKs suggest that the various mammalian cyclins and CDKs may take each other’s roles if necessary (5, 6).

According to the Aguda-Tang kinetic analysis, sharp switching behavior in CDK2 activity is predicted from the mutual-activation (between CDK2 and Cdc25A) and mutual-inhibition (between CDK2 and p27Kip1) as shown in the bottom right black box of Figure 2. Cdc25A removes an inhibitory phosphate from CDK2 thereby promoting the latter’s kinase activity; in return, the phosphatase activity of Cdc25A is upregulated by its phosphorylation carried out by CDK2. Such positively coupled cycles of phosphorylation and dephosphorylation possess an intrinsic instability that generates switching behavior (as shown in Ref. 7); this switch is further sharpened when coupled to

the mutual antagonistic interactions between p27Kip1 and CDK2. Several experimental observations are consistent with the Aguda-Tang model (8-10).

In quiescent cells, members of the retinoblastoma protein (pRB) family bind and inhibit proliferative transcription factors such as the E2Fs which are essential for the expression of several S-phase genes. Growth factors stimulate the synthesis of the D-type cyclins which bind and activate CDK4 and CDK6; these kinases then phosphorylate and inactivate pRB subsequently freeing E2F to induce the expression of cyclin E, cyclin A, Cdc25A, several members of ORC (origin recognition complex), etc. Cyclin E/CDK2 and cyclin A/CDK2 further contribute to the hyperphosphorylation of pRB (see Ref. 11 for review). The time lag in the activation of cyclin E/CDK2 after crossing the restriction point (as reported in Ref. 8 and in a model simulation discussed in Ref. 3) could be explained by an induction time associated with the build up of the positive feedback loop involving pRB, E2F, and cyclin E/CDK2 (see Figure 2).

< **Figure 2 here** >

2.2 Apoptosis Module

The apoptosis module involves cascades of activation of proteases of the caspase family (see Ref. 12 for a recent review). Members of this family include the initiator caspases (caspase-8, -9, -2, -10) and executioner caspases (caspase-3, -6, -7). The two major pathways that converge at active executioner caspases are summarized in the left black box of Figure 2 and in the review of Ref. 2. The extrinsic or membrane receptor-mediated pathway involves a complex called DISC (death-inducing signaling complex)

which forms upon ligand-receptor binding (e.g. TNF α and FasL ligands binding to corresponding receptors). For example, the accumulation of procaspase-8 in the DISC generates active caspase-8 molecules which are released from the complex to activate the executioner caspases. The extrinsic or mitochondrial pathway involves the formation of the 'apoptosome', a complex composed of cytochrome *c*, Apaf-1, and procaspase-9. Due to internal cellular stresses (e.g. DNA damage, reactive oxygen species, growth factor withdrawal) pro-apoptotic proteins (e.g. Bax, Bad) induce permeabilization of the mitochondria and subsequent release of cytochrome *c*. Formation of the apoptosome is followed by the activation of caspase-9 which promotes the activation of the executioner caspases. As shown in the apoptosis module in Figure 2, the extrinsic pathway feeds into the intrinsic pathway (e.g. via activation of Bid by caspase-8; Bid induces release of cytochrome *c* from mitochondria); moreover, a positive feedback loop is shown from the executioner caspases to the step that forms the apoptosome (representing the observation that executioner caspases induce an increased rate of cytochrome *c* release from mitochondria (13, 14). This positive feedback loop is thought to be an essential mechanism for the irrevocable switch into apoptosis (2).

3. THE E2F-MYC NODE

3.1 The E2F-Myc node, its regulation and targets

Shown in Figure 2 is the E2F-Myc transcriptional node that induces expression of several genes involved in S-phase entry as well as genes involved in apoptosis. E2F dimerizes with DP proteins while Myc dimerizes with Max in order to carry out their respective transcriptional activities. E2F-1 has been shown to upregulate c-Myc expression (15-17)

and c-Myc has been shown to induce transcription of E2F-1, E2F-2, and E2F-3 (18, 19). Thus, E2F-1 and c-Myc synergize to induce S-phase entry and apoptosis (2, 20).

A schematic representation of signal transduction pathways impinging on the E2F-Myc node is shown in Figure 2. One involves a pathway from cyclin D/CDK to the retinoblastoma protein (pRB). Several studies have shown that the Ras family of small GTPases are involved in the upregulation of synthesis or stabilization of some D-type cyclins (reviewed in Ref. 11). These cyclins bind and activate CDK4 or CDK6 which ultimately results to increased E2F transcriptional activity (because these CDKs phosphorylate pRB which then releases E2F). The significance of studying the pRB pathway is underlined by the fact that it is targeted for inactivation in at least 80% of sporadic human cancers (21). Chau and Wang (22) recently reviewed evidence that pRB could be a key protein in the regulation of a cell's life and death decisions. This role of pRB is directly associated with its inhibition of the E2Fs. The interplay between mitogenic signals and death signals (e.g TNF signaling) uses pRB as a key player; mitogenic signals inactivate pRB by phosphorylation via CDK activation, whereas death signals inactivate pRB by caspase-dependent degradation (see Ref. 22 for review).

A few of the major transcriptional targets of E2F-1 are shown in Figure 2. These include S-phase genes such as cyclin E, cyclin A, Cdc25A, and several members of the pre-replication protein complex formed prior to DNA replication (reviewed in Ref. 11). It is also interesting to note that there is a positive feedback loop involving the E2F-1 protein as a positive transcription factor for its corresponding gene (23). Among the pro-apoptotic genes induced by E2F-1 are Apaf-1 (a member of the apoptosome complex) and various caspases (24-26). Another E2F-1 target is ARF which indirectly stabilizes

p53. E2F-1 can also promote apoptosis, independent of its transcriptional activity, by binding with the p65 subunit of NF- κ B and inhibiting the latter's anti-apoptotic functions (27). In the presence of DNA damage, ATM, ATR and Chk2 kinases have been shown to phosphorylate E2F-1 resulting in the stabilization of this substrate (28).

Note that activation of cyclin D/CDK4/6 is not absolutely required for the activation of cyclin E/CDK2; this is because signaling pathways mediated by c-Myc can upregulate expression of proteins involved in CDK2 activation as well as in the downregulation of the CDK inhibitor p27Kip1 (29). Santoni-Rugiu et al. (29) have demonstrated that Myc is involved in a G1/S-promoting mechanism that is parallel to the pRB/E2F pathway. Myc is an immediate-early gene that is expressed soon after a cell's exposure to growth factors leading to the activation of tyrosine kinases such as Src and JAK that upregulate Myc (reviewed in Ref. 11). Myc's transcriptional targets which are involved in S-phase entry include cyclin D2 (30, 31), CDK4 (32, 30), cdc25A(33), E2F-2 (18), and cyclin E (34). Pro-apoptotic target genes include ARF and Bax as shown in Figure 2. The pathway leading to apoptosis is also favored by the suppression of the protein and RNA levels of Bcl-2 by c-Myc as well as by E2F-1 (35). Furthermore, it has been suggested (36) that c-Myc promotes apoptosis by repressing the expression of p21Cip1 which indirectly inhibits apoptosis via the mitochondrial pathway (37).

3.2 Coordinating S-phase and Apoptosis

A major question of interest is how the E2F-Myc node coordinates the initiation of S-phase and apoptosis; as an example of this coordination, E2F-1 first stimulates S-phase entry when its transcriptional activity is within some normal range but drives the

apoptosis program when overexpressed. Looking at Case I in Figure 1, it is important to remember that the arrow from the E2F-Myc transcriptional node to apoptosis indicates that apoptosis is potentiated and not necessarily triggered; could it be that a trigger for apoptosis is generated by an over-stimulated cell cycle? Is it also possible that the cell cycle mechanism itself generates a signal that somehow inhibits apoptosis so that the cell is not in danger of accidentally killing itself when everything else is normal? And how do the different cases in Figure 1 interact to determine cell fate? These questions were addressed in a recent paper of Aguda and Algar (2). It is proposed in this paper that the complexity of the network of molecular interactions can be reduced by subdividing the network into functional modules and analyzing module-module interactions. These modules and their interactions are shown in Figure 3. The module-module interactions are labeled **a**, **b**, **b'**, and **c**. Interaction **a** includes positive feedback loops involving the transcriptional node and the S-phase module; examples would be the loop E2F-CyclinE/CDK2-RB-E2F and the autocatalytic loop involving E2F-1. Aguda and Algar (2) noted the importance of the negative feedback **b** from the cell cycle module to the signaling pathway upstream of the transcriptional node; as the computer simulations in their paper demonstrate, this negative feedback permits a window or a range of transcriptional activities for normal cell cycles to exist by inhibiting apoptosis. The existence of a negative feedback loop involving the Ras/Raf/MEK/ERK pathway can be supported by experimental evidence (38- 40) of multiple peaks of activities of Ras and/or MEK/ERK; these peaks look like damped oscillations which are indicative of negative feedback in a dynamical system. Another negative feedback is the interaction **b'** between the cell cycle module and the transcriptional node; the phosphorylation of DP-1 (a partner

of E2Fs) by CDK2 can turn off E2F-1 transcriptional activity (41, 42). Lastly, the feedforward type of interaction **c** in Figure 3 is a direct way of triggering apoptosis when the cell cycle ‘overheats’; experimental evidence for this interaction has been reported (43-45).

< **Figure 3 here** >

4. THE p53 NODE

The p53 protein is a DNA sequence-specific transcription factor that regulates the expression of certain genes associated with the induction of apoptosis, cell cycle arrest, prevention of new blood vessel formation, and accelerated DNA repair. The induction of apoptosis is widely accepted as the primary role of p53 in tumor suppression. The levels of p53 in normal cells are low but rapidly increases upon exposure to DNA-damaging stresses including ultraviolet light and intense oncogenic signaling. The p53 gene is mutated in about half of known human cancers while a majority of the remaining cases are due to dysfunctional regulation of an otherwise normal p53 protein (see Refs. 20, 46-48 for reviews).

The p53 control node shown in Figure 4 symbolizes a set of active transcription factor complexes involving p53 that directly affect the expression of target genes some of which are shown in the figure. Signaling to the p53 node includes all upstream conditions and processes (e.g. exposure to ultraviolet light, presence of DNA damage) that cause elevated rates of p53 synthesis, covalent modifications of the protein, DNA binding and activation of p53-mediated transcription. Post-translational modifications

(i.e. phosphorylation, acetylation, sumoylation) that regulate p53 activity are also considered processes signaling to the node. Many details of these regulatory pathways are already known, and comprehensive reviews of the current state of knowledge in the field are available (e.g. Refs. 46, 49, 50) and will not be duplicated here. The primary aim here is to interpret the significance of these pathways in relation to their role in deciding whether the cell dies or survives. The interpretation is based on significance of the cycles present in the regulatory network. Figure 5 summarizes some of the positive and negative feedback loops in the p53 regulatory network. Harris and Levine (51) recently reviewed the various positive and negative feedback loops in the p53 regulatory network.

< **Figure 4 here** >

< **Figure 5 here** >

4.1 The p53-Mdm2-ARF network and negative feedback loops

As shown in Figures 4 and 5, the regulation of p53 activity involves several coupled negative feedback loops; one of these is a 2-cycle involving Mdm2 and p53, and another is a 3-cycle involving p53, ARF, and Mdm2. In the 2-cycle, Mdm2 binds and inhibits p53 by blocking a transactivation domain of p53 and by promoting the ubiquitination of p53 leading to proteosomal degradation; on the other hand, p53 induces the expression of the *mdm2* gene by binding to this gene's promoter region. This negative feedback loop

between p53 and Mdm2 has been offered as the origin for observed oscillations in p53 activity (52, 53).

The transcription of the p14/19/ARF gene is negatively regulated by p53 (reviewed in Ref. 51). In turn, ARF binds to and represses the ubiquitin ligase activity of Mdm2 (54). ARF is not expressed in normal proliferating cells but is rapidly expressed in response to many oncogenes such as c-Myc, E2F-1, Ras and β -catenin; this is why ARF is considered as one of the most important 'sensors for aberrant proliferative signalling' (20).

As shown in Figure 5, p53 induces transcription of SIAH-1, a ubiquitin ligase that targets β -catenin for degradation (51). A negative feedback loop is thus formed by the pathway represented by the sequence p53-(SIAH-1)-(β -catenin)-ARF-Mdm2-p53. This links the β -catenin signaling pathway to the p53 network. Other negative feedback loops shown in Figure 5 are p53-p21-CDK2-Mdm2-p53 and p53-(WIP-1)-p38MAPK. The gene p21Cip1 which produces a CDK (cyclin-dependent kinase) inhibitor is one of the first identified transcriptional targets of p53. As shown in Figure 5, CDK2 inhibits Mdm2 by phosphorylation; interestingly, this inhibitory phosphorylation is counteracted by the phosphatase PP2A in complex with cyclin G which is rapidly transcribed after p53 activation in many cell types (reviewed in Ref. 51). This gives a picture of Mdm2 regulation by phosphorylation-dephosphorylation steps which are influenced by p53 via p21 (resulting to inhibition of the phosphorylation step) and via cyclin G (which enhances the dephosphorylation step).

The Ras/MAPK signaling pathways are linked to the p53 regulatory network as shown in Figure 5. Activation of p53 partly involves its phosphorylation by p38MAPK

on certain serine residues (reviewed in Ref. 51). The protein p38MAPK itself is activated by phosphorylation which is reversed by the Wip-1 phosphatase.

4.2 Positive feedback loops in p53 regulation

Shown in Figure 5 is a positive feedback loop represented by the sequence p53-PTEN-PIP3-Akt-Mdm2-p53. PTEN is considered a tumor suppressor whose transcription is induced by p53 in some cell types (reviewed in Ref. 51). PTEN is a lipid phosphatase that dephosphorylates PIP3 (phosphatidyl inositol-3,4,5-triphosphate). PIP3 is required for the recruitment of Akt to the plasma membrane where it gets phosphorylated and activated. In addition, p53 represses the catalytic subunit of PI3K (phosphatidylinositol 3-kinase) which catalyzes the formation of PIP3 (55). Akt is a survival factor because it phosphorylates and subsequently inhibits pro-apoptotic proteins such as Bad and caspase-9. Akt also phosphorylates Mdm2 which subsequently translocates to the nucleus where it inhibits p53. The positive feedback loop between p53 and Akt is of the mutual antagonism kind: high p53 activity (apoptosis) means low Akt activity (low survival), and vice versa. Indeed, such mutual antagonism could provide a mechanism for switching between the two cell fates of survival and death.

The picture becomes more complicated when the participation of another important kinase, glycogen synthase kinase-3 β (GSK-3 β) is considered. The substrates of this kinase regulate cell proliferation and metabolism (56, 57). Shown in Figure 6 is the interlocking pathways of p53, Akt, GSK-3 β , and β -catenin. This figure shows various positive feedback loops in addition to that between Akt and p53 discussed in the preceding paragraph. GSK-3 β and p53 have been shown to interact directly (58) and that

this interaction augments the activities of both proteins (48) (as symbolized by the double arrows in Figure 6). GSK-3 β phosphorylates and subsequently inactivates proliferative factors such as cyclin D, c-Myc, and β -catenin, among others. True to its role as a survival factor, Akt can phosphorylate and inactivate GSK-3 β as this kinase has been shown to trigger apoptosis when in excess (59). The pathway of activation of Akt from β -catenin could involve increased expression of WISP-1 (Wnt-induced-secreted-protein-1) (reviewed in Refs. 4, 60).

The question remains regarding the role of the positive feedback loops involving p53. It was stated above that the positive feedback between Akt and p53 is a good candidate mechanism for a switch between survival and apoptosis. The positive feedback loop between GSK-3 β and p53 could be a mechanism for favoring the apoptosis pathway. The positive loop (see Figure 6) is another candidate for a switching mechanism between survival and death because it could be interpreted as a toggle switch between Akt (survival) and GSK-3 β (apoptosis). The interactions among these positive loops (as well as between negative and positive feedback loops) are difficult to understand using mere intuitive reasoning and it would be interesting to see what computational modeling can contribute to future understanding of this complex network.

< **Figure 6 here** >

4.3 Are there critical determinants of p53-mediated decision on life and death?

If there is a critical determinant of a p53-mediated decision on life or death of a cell, it will surely depend on the cellular context such as the presence of survival signals and cell genotype (48). For example, Myc has been suggested as a critical determinant between p53-mediated cell cycle arrest and apoptosis (47). Without Myc, cells exposed to ultraviolet light undergo growth arrest primarily due to the activation of the CDK inhibitor p21 (61, 62). Myc (via recruitment by Miz-1) effectively blocks p21 induction by p53 and tilts the decision towards apoptosis instead of growth arrest (63). In human colorectal cancers, the balance between p21 and PUMA appears to be critical in deciding to arrest or die in response to exogenous p53 (reviewed in Ref. 47). Another possible critical determinant is E2F-1 which cooperates with p53 in various ways, e.g. by direct E2F1-p53 association, and by binding to adjacent promoter sites of pro-apoptotic genes as best illustrated by the case of Apaf-1 (24). The authors of this chapter think that identification of critical determinants of p53-mediated life or death decisions will ultimately depend on understanding the feedback loops between p53-induced apoptotic pathways and survival pathways (e.g. the Akt pathway).

5. THE NF- κ B NODE

5.1 The NF- κ B node, its regulation and targets

NF- κ B is a family of inducible transcription factors that are key responders to changes in cellular environment (64). They play their most important role in the immune system by producing chemokines that trigger innate immune responses. The family consists of p50 (NF- κ B1), p52 (NF- κ B2), p65 (RelA), RelB and c-Rel. These members form dimers in various combinations in order to carry out their transcriptional activities. The

prototypical NF- κ B complex is the heterodimer p50/p65. Each member protein contains a Rel-homology domain that is involved in binding to a DNA sequence motif called the κ B site, in dimerization with other family members, and in binding to I κ B which inhibits nuclear translocation of NF- κ B. Some of the transcriptional targets of NF- κ B are involved in cell proliferation (e.g. cyclin D1 and Myc), cell survival (e.g. Bcl-X_L, IAPs), angiogenesis (e.g. VEGF, IL-8), and the immune response (e.g. MHC-1, MHC-II). Malfunction of NF- κ B has been implicated in various hematological disorders, breast cancer, and many other cancers and human diseases (for recent reviews see Refs. 65 and 66). Connections between inflammation and cancer have also been reviewed recently (67, 68).

The canonical pathway of NF- κ B activation is initiated by upstream signals that activate a macromolecular complex called the ‘signalsome’ (69). The upstream signals include cytokines (e.g. TNF α , LPS, CD40L), reactive oxygen species, viral and bacterial products (70). The ‘signalsome’ is an IKK complex composed of IKK α , IKK β and IKK γ (NEMO). IKK β is the principal kinase in the signalsome that phosphorylates I κ B leading to the latter’s degradation; free NF- κ B then rapidly translocates to the nucleus.

Non-canonical pathways are initiated by agonists such as CD40L, BAFF, and RANKL that induce the activation of a protein kinase called NIK (NF- κ B inducing kinase) (64, 70). NIK phosphorylates a dimer of IKK α which initiates proteolytic processing of p100 to p52. Subsequently, p52 along with its dimer partner (principally RelB) translocates to the nucleus.

Signaling to the NF- κ B node involves both positive and negative feedback loops as shown in Figure 7. The negative feedback is due to NF- κ B inducing transcription of

I κ B which inhibits NF- κ B in return (71-73); more specifically, inside the nucleus, the newly synthesized I κ B α protein binds NF- κ B resulting to the export of the complex out of the nucleus (74, 75). Interestingly, NF- κ B is also known to upregulate the synthesis of proteins that activate NF- κ B, thus forming a positive feedback loop (see Figure 7); examples are the proteins activating upstream signals of the canonical pathway such as TRAF1/2 (76), CD40 (77), and CD40L (78).

< **Figure 7 here** >

5.2 Anti-apoptotic, pro-apoptotic, and proliferative pathways from NF- κ B

NF- κ B protects the cell from apoptosis through induction of anti-apoptotic genes such as Bcl-X_L (79), Bfl-1/A1 (80), cFLIP (81) and IAPs (76, 82). These genes inhibit apoptosis in various ways. Examples include IAPs (Inhibitors of Apoptosis) inhibiting caspase-3, -6, -7 and -9; cFLIP inhibiting the apoptosome; and Bcl-2 inhibiting the release of cytochrome *c* from mitochondria.

On the other hand, NF- κ B has been shown to target pro-apoptotic genes such as FasL (83), DR6 (84), and caspase-11 (85). Characteristics of the activating stimulus of NF- κ B may determine whether anti-apoptotic or pro-apoptotic genes would be induced (86). Interestingly, both the pro- and anti-apoptotic functions of NF- κ B could occur in the same cell (87), and it may well be that the role of NF- κ B in a cell's decision to live or die is the net result of these opposite apoptotic functions. What then determines the net effect of NF- κ B on apoptosis? Differential regulation of apoptosis between different member proteins of the NF- κ B family is possible, e.g. p65/p50 inhibits transcription of

the Bax gene while p50/p50 upregulates it (88). In addition, relationships between NF- κ B family members and apoptotic p53 may play some role. There is evidence showing that NF- κ B and p53 compete for the same coactivator, CBP/p300, effectively inhibiting each other (89-93). On the other hand, it has been shown that p65/p50 induces p53 transcription (94-96) (later shown to require AP-1 and Myc/Max transcription factors (97)).

Also shown in Figure 7 is the interaction between the NF- κ B and the E2F-Myc nodes. NF- κ B induces transcription of Myc while E2F-1 represses the activation of NF- κ B by inhibiting the degradation of I κ B (98) or by binding to the p65 subunit (27), or through some other unknown proteins (99). The influence of NF- κ B on apoptosis and the cell cycle is therefore intertwined with that of the E2F-Myc transcriptional node as discussed in a previous section.

NF- κ B regulates the cell cycle via induction of cyclin D1 (100) and c-Myc (101), factors that promote G1-to-S progression. There is also evidence indicating the presence of NF- κ B binding sites upstream of cyclin D2 (102) and cyclin D3 (103) genes. Studies (104-106) have shown that NF- κ B represses GADD45 (- α , - γ), which in turn promotes cell survival by blocking JNK-induced apoptosis. Mak and Kultz (107) further showed that the various GADD45 isoforms (- α , - β , - γ) promote early stages of apoptosis but inhibit mitosis. A study by Taylor and Stark (108) also showed that p53 induces G2/M arrest partly through GADD45 proteins.

6. CONCLUDING REMARKS

This chapter has presented a perspective for viewing the complexity of intracellular molecular networks associated with a cell's response to growth and death factors in its environment. These responses are coordinated by certain transcription factors which are referred to in this chapter as 'control nodes' because each represents a meeting point of signalling pathways (inputs) and pathways (outputs) that diverge to the cell cycle and apoptosis machineries. The complexity of these molecular networks is reduced by subdividing them into modules and by assuming that the module-module interactions in a network are sufficient to understand the essential behaviour of the entire network.

Only three transcriptional control nodes were discussed in this chapter but there are others that can also affect both the apoptosis and cell cycle programs, including the Smad family of transcription factors, FOXO, etc. (see Ref. 4). The E2F-Myc node (primarily represented by the E2F-1 and c-Myc heterodimeric transcription factors) was discussed above to illustrate transcription factors that potentiate both the initiation of the cell cycle and the apoptosis programs. It may be surprising at first why such a possibility exists at all given that cell cycle and death are diametrically opposite cell fates; but, as discussed above, such a nodal link does provide a mechanism for the organism to carry out the fragile balance between cell proliferation and death (e.g. during its multicellular development). As explicitly analyzed by Aguda and Algar (2) through mathematical modelling, the module-module interactions allow a number of cell fates to be orchestrated sequentially – namely, quiescence, cell cycling, and death - as growth factor signalling intensities increase. The feedback loops in these modular interactions played crucial roles in the behavioural transitions of the system.

The second transcriptional node discussed was p53, a protein of high significance because of its tumor suppressor activity. As summarized in Figure 5, the core p53-Mdm2-ARF regulatory module is linked to the Akt, p38MAPK, and β -catenin signalling pathways that involve feedback loops. It was pointed out that the mutual antagonism between p53 and Akt is a good candidate for a switching mechanism that toggles between cell death and survival. How this toggle switch is controlled by the associated pathways would be an interesting topic for kinetic modelling.

The NF- κ B family of transcription factors was used as an example of a transcriptional control node that inhibits apoptosis but promotes the cell cycle. However, as discussed above, it is now known that NF- κ B can also induce expression of pro-apoptotic genes, and it is still an open question how these opposing contributions to apoptosis are sorted out in a given cell. The positive and negative feedback loops involved in NF- κ B signalling (see Figure 7) have been the subject of recent mathematical modelling of temporal control of gene expression (109, 110).

Lastly, the three transcriptional nodes discussed here interact in various ways as shown in Figures 2, 4, and 7. It is probable that only a subset of these interactions is realized for any given set of extracellular stimulus. The real challenge is to understand the logic of these interactions, their control, and to predict whether the cell proliferates, survives or die.

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FIGURE CAPTIONS

Figure 1. Transcriptional nodes (symbolized by a circle, a triangle, a square, and a hexagon) are classified according to how they affect the cell cycle and apoptosis. Arrows represent ‘induction’ while hammerheads represent ‘inhibition’. Associated intracellular signalling pathways that activate these nodes are shown.

Figure 2. The E2F-Myc node is the top-most black box which encapsulates the synergy between the E2F and Myc transcription factors. Dotted lines indicate transcriptional activation (with arrow heads). Regulation of the E2F-Myc node activity includes both Rb-dependent and Rb-independent pathways as represented by the two signalling routes shown in the figure. The right-most black box represents a minimal module for the regulatory network involved in CDK2 activation in S-phase entry. Solid lines mean post-transcriptional interactions with an arrow indicating ‘activation’ and a hammerhead denoting ‘inhibition’. The left-most black box represents the apoptosis module involving cascades of activation of the caspases. See text for more details.

Figure 3. Case I of Figure 1 including module-module interactions. Arrows indicate activation while hammerheads indicate inhibition. This is the modular model analyzed by Aguda and Algar (Ref. 2). The signalling, apoptosis, and cell cycle modules were assumed to possess ultrasensitive kinetics (as shown by the curves inside the rectangular boxes). The transcriptional node (circle) was not assumed to have that kinetics.

Figure 4. The p53 transcriptional factor promotes expression of certain pro-apoptotic genes (e.g. FasL, Bax, etc.), of the CDK inhibitor p21, and represses transcription of certain anti-apoptotic genes (e.g. Bcl-XL and Bcl-2). The core regulatory network involving Mdm2 and ARF is shown, as well as the links of this network to Akt and β -catenin. Interactions among the NF- κ B and E2F-Myc nodes are discussed in the text. Dotted lines represent transcriptional processes while solid lines are post-translational interactions. Arrows mean ‘activation’ and hammerheads mean ‘inhibition’.

Figure 5. Some of the feedback loops coupled with the p53-Mdm2-ARF core regulatory network as reviewed by Harris and Levine (Ref. 51). See text for details.

Figure 6. Some of the positive feedback loops involving p53. Mutual antagonism between p53 (pro-apoptotic) and Akt (pro-survival), involving PTEN and GSK-3 β , could provide a switching mechanism between cell survival and apoptosis. On the other hand, repression of β -catenin by p53 and GSK-3 β could reduce β -catenin-Akt mediated tumorigenesis. See text for details. (Figure is modified from Figure 6 of Ref. 48)

Figure 7. Overview of the NF- κ B control node. Upstream stimuli activate NF- κ B via two groups of pathways. In the canonical pathway, activated IKK complex triggers degradation of I κ B leading to activation of NF- κ B; non-canonical pathways involve NIK activation of IKK α dimer to produce NF- κ B heterodimers. NF- κ B-induced transcription of I κ B generates a negative feedback while NF- κ B-induced transcription of TRAF1/2, CD40, and CD40L results in positive feedback loops. As shown on the left-hand side of the diagram, NF- κ B may induce expression of both anti-apoptotic and apoptotic genes.

NF- κ B's role in the cell cycle is exemplified by promoting the expression of genes such as cyclin D and gadd45 as shown on the right-hand side of the figure. See text for more details.

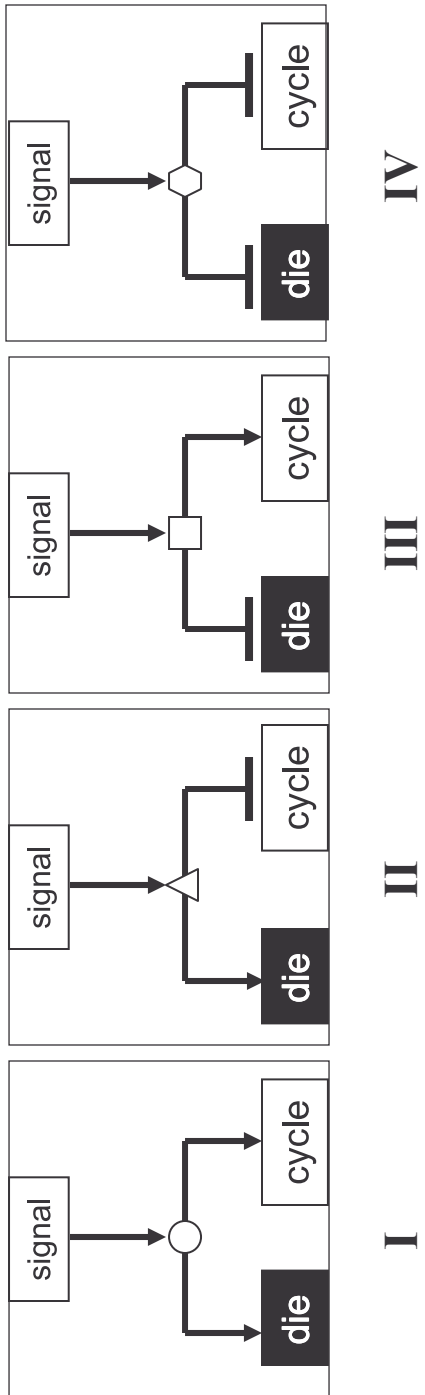


Figure 1 (Aguda et al.)

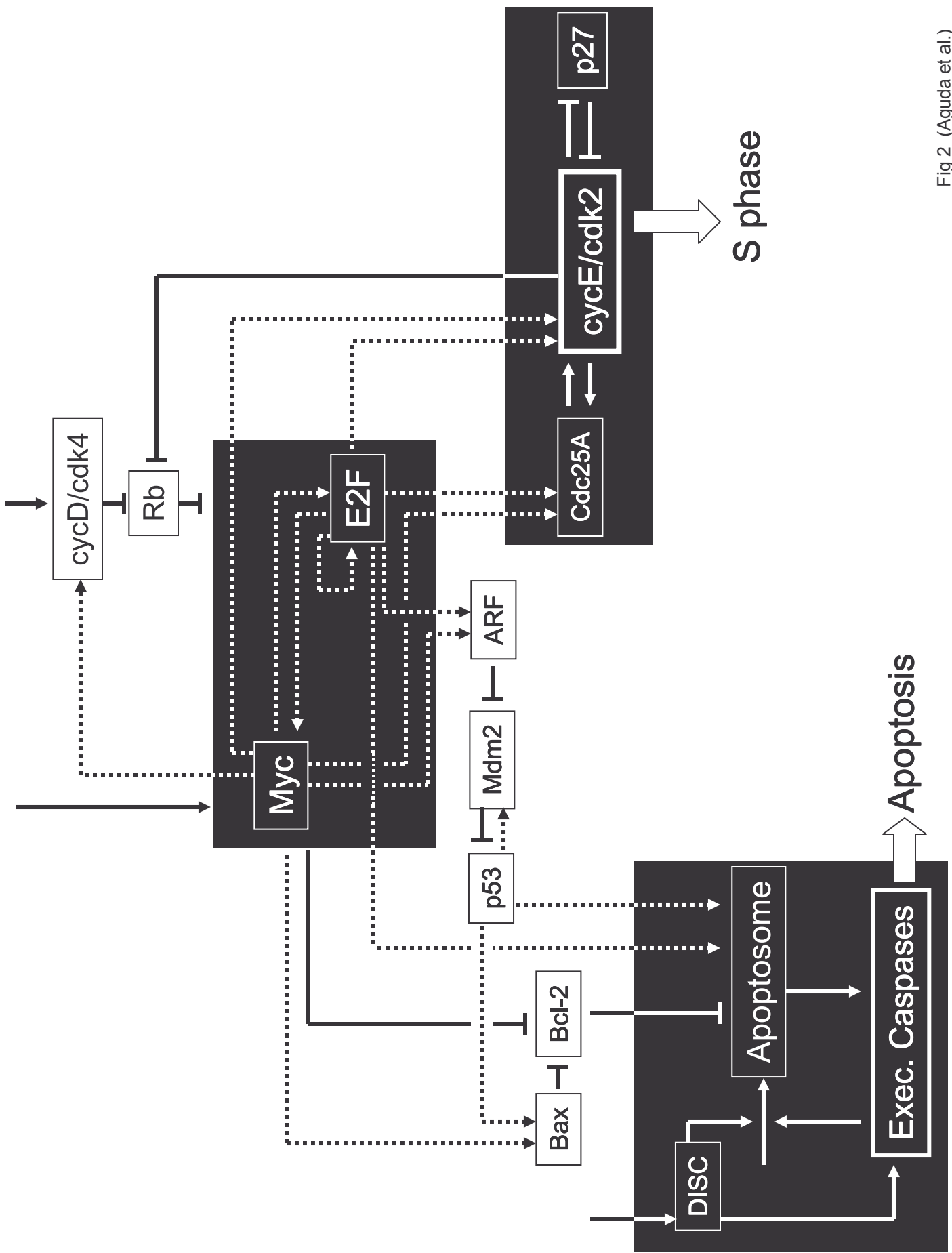


Fig 2 (Aguda et al.)

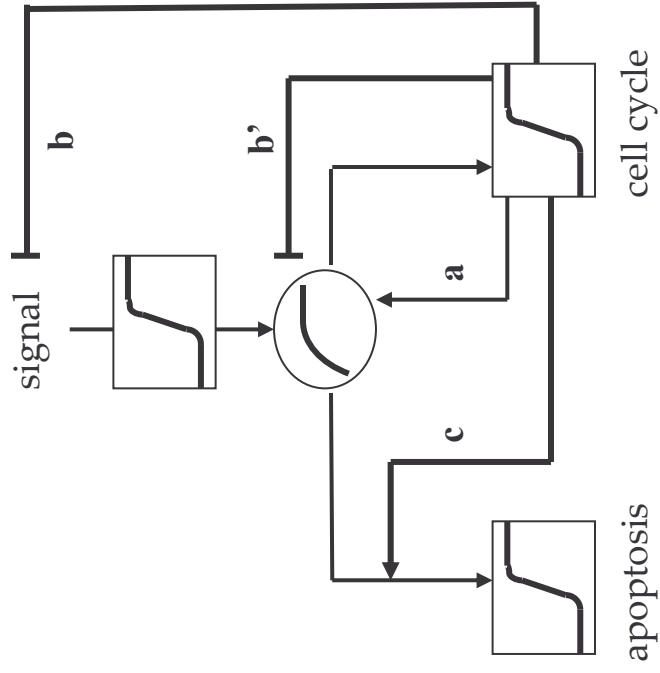


Figure 3 (Aguda et al.)

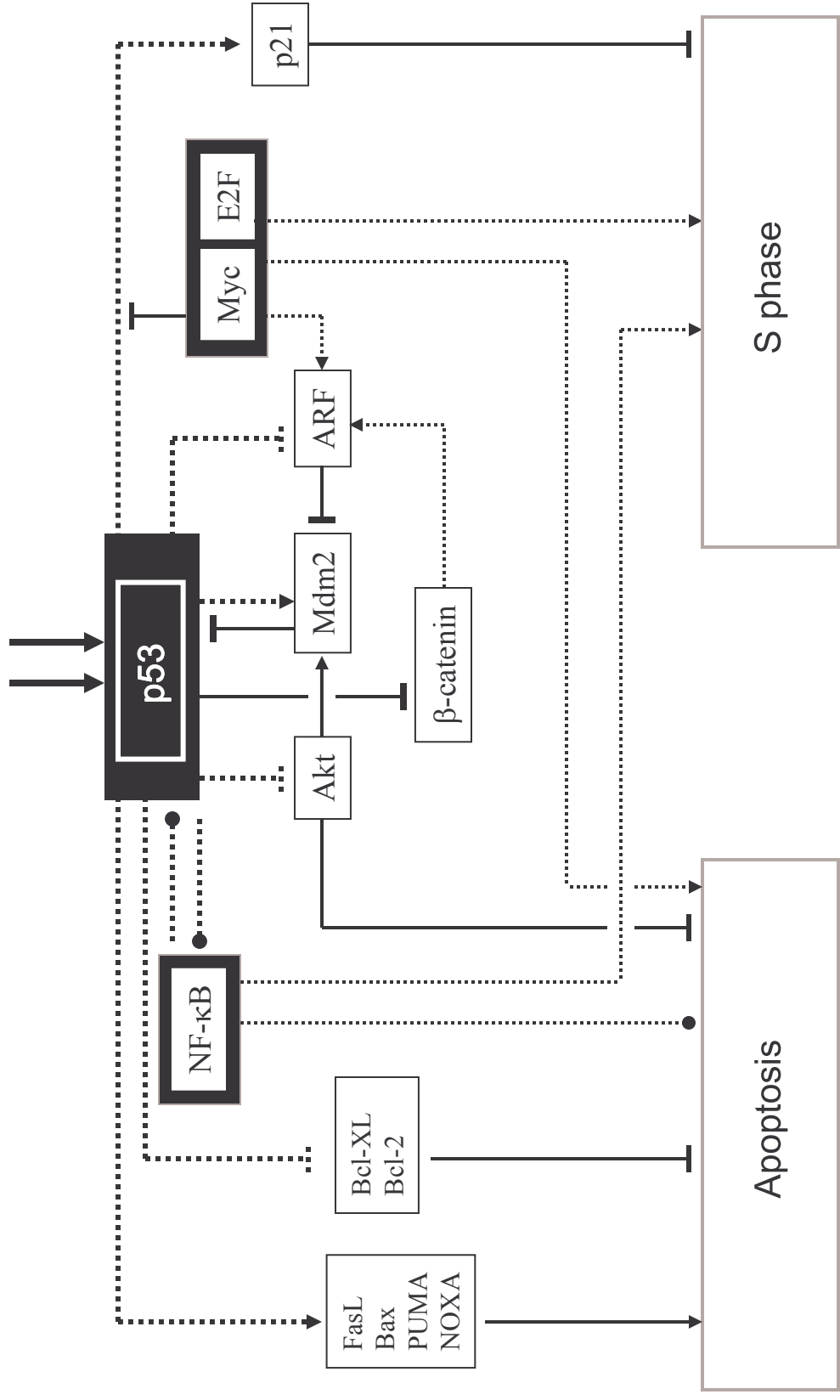


Figure 4 (Aguda et al.)

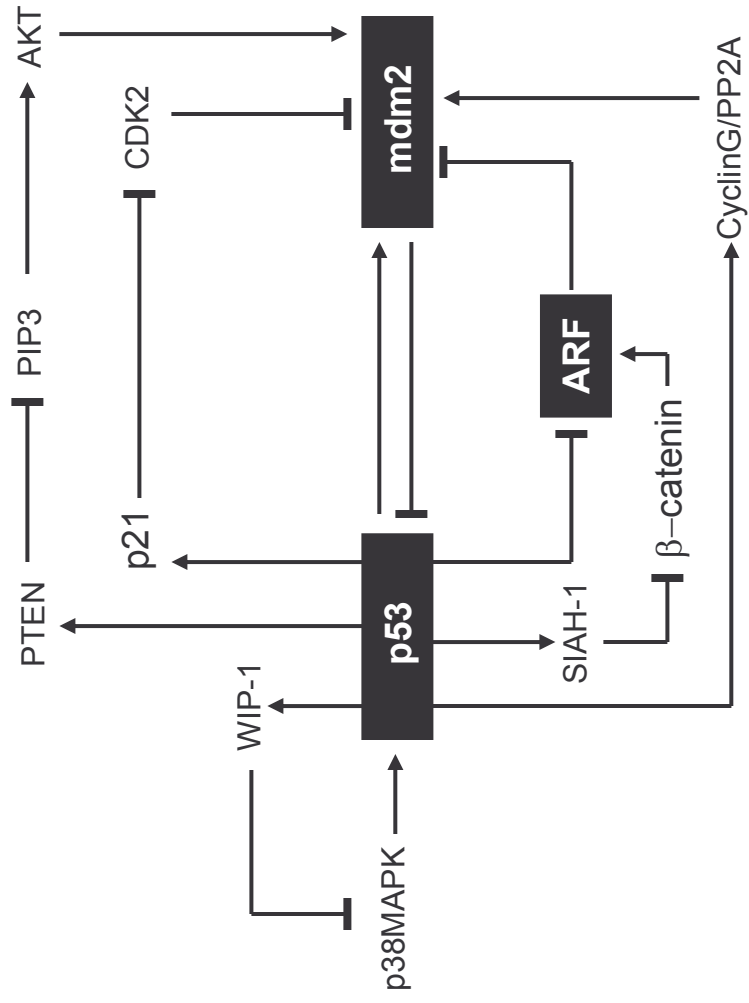


Figure 5 (Aguda et al.)

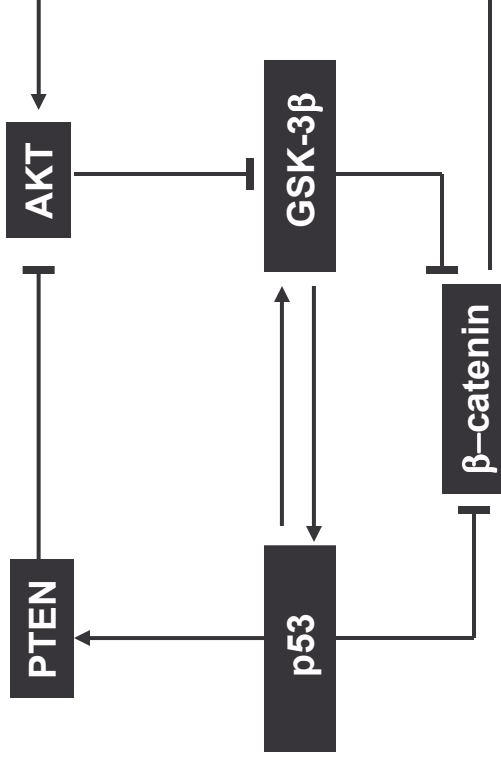


Figure 6 (Aguda et al.)

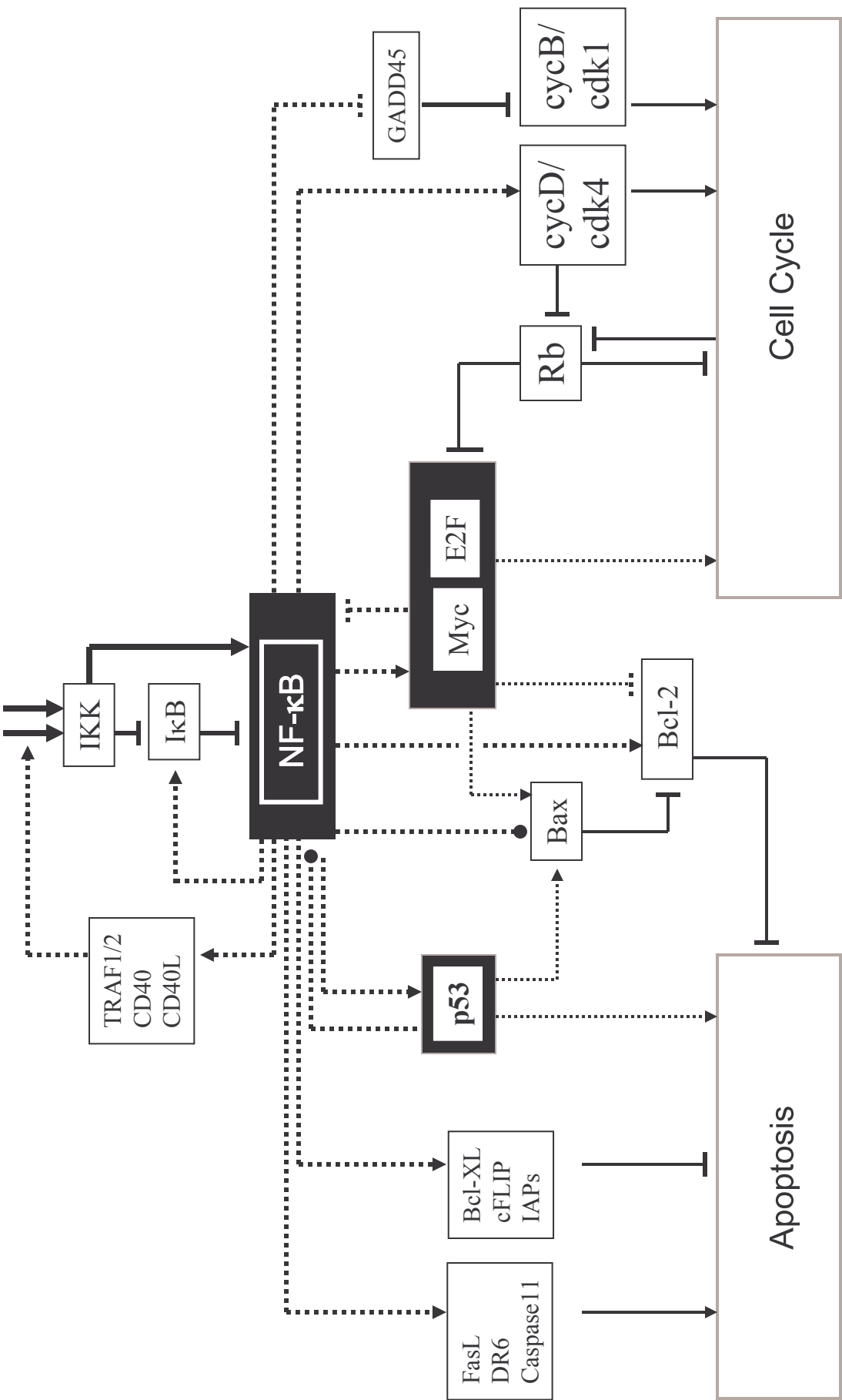


Figure 7 (Aguda et al.)