The retinoblastoma tumor suppressor, pRb, restricts cell-cycle progression mainly by regulating members of the E2F-transcription-factor family. The Rb pathway is often inactivated in human tumors, resulting in deregulated-E2F activity that promotes proliferation or cell death, depending on the cellular context. Specifically, the outcome of deregulated-E2F activity is determined by integration of signals coming from the cellular DNA and the external environment. Alterations in cell proliferation and cell-death pathways are key features of transformed cells and, therefore, an understanding of the variables that determine the outcome of E2F activation is pivotal for cancer research and treatment. In this review, we discuss recent studies that have elucidated some of the signals affecting E2F activity and that have revealed additional E2F targets and functions, thereby enriching the understanding of this versatile transcription-factor family.

Introduction
Members of the E2F family of transcription factors are downstream effectors of the tumor suppressor pRB and are considered to have a pivotal role in controlling cell-cycle progression. Initially, studies revealed that E2Fs determine the timely expression of many genes required for entry into and progression through the S phase of the cell cycle. However, it has become clear that transcriptional activation of S-phase-related genes is only one facet of E2F activity; it is now known that E2Fs both transactivate and repress gene expression. Furthermore, E2Fs function in a wide range of biological processes, including DNA replication, mitosis, the mitotic checkpoint, DNA-damage checkpoints, DNA repair, differentiation, development and apoptosis [1–4]. Considering this extensive list, it is perhaps not surprising that the best-studied member of the family, E2F1, exhibits both oncogenic and tumor-suppressive activities.

E2F transcriptional activity is modulated by multiple mechanisms, the best known being interaction with the product of the retinoblastoma (Rb) tumor-suppressor gene, pRB [5]. This association not only inhibits the ability of E2F to transactivate but, also, actively represses transcription through the recruitment of various chromatin modifiers and remodeling factors to the promoters of E2F-responsive genes. These co-repressors include histone deacetylases (HDACs), histone methyltransferases and DNA methyltransferases [4]. Formation of pRB–E2F complexes is cell-cycle regulated, with dissociation leading to derepression and activation of E2F-regulated genes and S-phase entry.

Deregulated-E2F activity occurs in the vast majority of human tumors through several different mechanisms. These include functional loss of pRB; amplification of cyclin D, which promotes phosphorylation of pRB; loss of p16, a cyclin-dependent kinase inhibitor that inhibits the phosphorylation of pRB; and expression of the human papillomavirus (HPV) oncoprotein E7, which disrupts pRB–E2F complexes [6].

In mammals, the E2F family comprises eight genes (E2F1–8), which give rise to nine distinct proteins. These include E2F3a and E2F3b, which are generated by the use of alternative promoters [5]. All family members contain a DNA-binding domain and E2F1–5 have a transactivation domain that enables activation of gene expression. A short amino-acid stretch that mediates binding of pRB is embedded within the transactivation domain and, thus, this domain also functions in repression of gene expression. E2F1–6 contain, in addition, a dimerization domain that is required for their interaction with a member of the dimerization-partner family (DP1–DP4). This interaction enables them to bind DNA and function as transcriptional regulators. E2F7 and E2F8 do not heterodimerize with DP-family members and can bind DNA as homodimers or as E2F7–E2F8 heterodimers [7,8]. E2F-family members have been categorized into subfamilies based on their transcriptional activity, structure and interaction with pRB-family members, p107 and p130. E2F1, E2F2 and E2F3a, which interact only with pRB, constitute one subfamily and are often referred to as the ‘activator E2Fs’, because they are believed to function mainly in activating gene expression. However, this classification is probably an over-simplification because DNA microarray studies show that activation of the so-called activator E2Fs leads to repression of almost as many genes as they activate. E2F4–8 function mainly in repression of gene expression and are often referred to as the ‘repressor E2Fs’.

A somewhat puzzling feature of activator E2Fs, and in particular E2F1, is the ability to induce the two seemingly contradictory processes of cell proliferation and apoptosis. Recent studies shed some light on a long-lasting question: how does E2F1 ‘decide’ whether to induce cell proliferation or cell death? These studies show that signals from damaged DNA direct E2F to apoptosis, whereas signal-transduction pathways, such as the phosphatidylinositol 3 kinase (PI3K)–protein kinase B (Akt) and epidermal-growth-factor receptor (EGFR)–ras pathways, inhibit...
E2F-induced apoptosis. These findings are of clinical relevance to the treatment and prognosis of cancer patients. In addition, recent studies identify novel functions of E2F, demonstrating that it regulates signal-transduction pathways and autophagy, a pathway that has a dual role in cell survival and cell death. The relevance of these novel activities of E2F to cancer development is currently being studied. In this review, these recent developments are discussed, focusing on the activator E2Fs.

**E2F and cell proliferation**

In quiescent cells, the activator E2Fs are bound to and repressed by pRB. Mitogenic stimuli that elevate cyclin-D levels promote phosphorylation of pRB by cyclin D–cyclin-dependent kinase (CDK) 4 or cyclin D–CDK6 complexes, which prevents pRB from binding E2Fs (Figure 1). Consequently, genes repressed by pRB–E2F complexes are derepressed and the now-free E2Fs also activate gene expression. Many of the upregulated genes encode proteins involved in DNA replication and cell-cycle progression, such as DNA polymerases, minichromosome maintenance complex components (MCMs), cdc6 and cyclin E. In line with the regulation of many proliferation-related genes by E2F, overexpression of E2F1, E2F2 or E2F3a induces quiescent immortalized cells to enter S phase [5]. These findings underlie the well-established role of E2Fs as pro-proliferative factors. However, E2F1 overexpression in primary fibroblasts does not lead to S-phase entry, but instead promotes senescence [9,10]. This indicates that immortalization is required for deregulated E2F1 to promote ectopic S-phase entry and uncontrolled cell proliferation. Moreover, these data imply that non-immortal cells might respond to ectopic-E2F expression by inducing senescence (or apoptosis, as discussed later) to inhibit tumorigenesis.

Different activator E2Fs seem to have partially overlapping, but distinct, roles in the regulation of cell proliferation. For instance, both E2F1 and E2F3 are required for cell-cycle entry, but only E2F3 is required for continued cell proliferation [11]. Combined loss of E2F1, E2F2 and E2F3 completely abolishes the ability of cells to progress through the cell cycle and proliferate [12]. Cells lacking E2F1, E2F2 and E2F3 exhibit impaired expression of E2F target genes that are essential for cell-cycle progression [12], thereby indicating that lack of activation of crucial E2F targets leads to cell-cycle defects. However, cells deficient in E2F3 exhibit derepression of the tumor suppressor Arf [13]. The Arf gene encodes a protein that stabilizes and activates p53 by negating the effects of the E3 ubiquitin ligase Mdm2, which otherwise promotes p53 degradation. Therefore, derepression of Arf in cells lacking E2F3 leads to activation of p53 and cell-cycle defects, in particular a defect in re-entering the cell cycle from quiescence. These abnormalities of E2F3-deficient cells are rescued by losing Arf [13]. Similarly, a recent study demonstrates that cells lacking E2F1, E2F2 and E2F3 exhibit p53 activation; the responsiveness of these cells to normal growth signals and expression of E2F target genes can both be restored by p53 loss [14]. E2F transcriptional-repressor complexes are crucial downstream targets of ARF (alternative reading frame of cyclin-dependent-kinase inhibitor 2A) and p53-induced proliferative arrest [15]. Based on these data, it is

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**Figure 1.** Upstream signals direct E2F1 to proliferation or apoptosis. Mitogenic signals and DNA damage activate different signaling pathways that determine whether E2F1 activity will induce transcription of proliferative or apoptotic genes. Mitogenic stimuli elevate cyclin-D levels, leading to repression of pRB activity via phosphorylation by cyclin D–CDK4 or cyclin D–CDK6 complexes. Subsequently, E2F1 is free to activate proliferative or apoptotic genes. When mitogenic signals also switch on the PI3K–Akt pathway or, in some situations EGFR–Ras–Raf signaling, the apoptotic activity of E2F1 is inhibited and it induces mainly proliferation. In response to DNA damage, signals direct E2F1 to activate its apoptotic target genes: pRB acetylation specifically releases E2F1, and not other E2Fs, to enable induction of apoptosis; E2F1 is phosphorylated by ATM and Chk2 and also acetylated and these modifications direct it preferentially to its apoptotic target genes. In addition, binding of Jab1, an E2F pro-apoptotic co-factor, enhances the apoptotic activity of E2F1. Conversely, SirT1 is induced after DNA damage and it can inhibit the apoptotic functions of E2F1.
hypothesized that, in addition to activating pivotal cell-cycle-related genes, E2F1, E2F2 and E2F3 regulate cell proliferation by controlling the ARF–p53 axis, which, in turn, induces E2F-mediated transcriptional repression (Figure 2). Such a functional link between activator E2F and repressor E2F is supported also by Drosophila melanogaster genetic studies demonstrating that many of the cell-cycle-progression defects and proliferation defects of flies lacking the activator E2F, dE2F1, can be suppressed by concomitant loss of the repressor E2F, dE2F2 [16].

Crossing the restriction point – E2F as a bistable switch

The concept of the restriction point, formulated in 1974, is that there exists a point in G1, usually 2–3 hours before S-phase entry, when commitment occurs and cells no longer require external stimuli to complete the cell cycle. The restriction point seems to be fundamental for normal differentiation and tissue homeostasis and is deregulated in practically all human tumors. A recent study demonstrates that of the first time a pivotal role for E2F in the restriction point, showing that the Rb–E2F pathway constitutes a bistable switch that controls this crucial point in the cell cycle [17]. This study shows that once there is a sufficient mitogenic stimulus, E2F is activated and maintains this ‘ON’ state independently of additional stimuli. Thus, the Rb–E2F pathway converts graded growth-stimulatory signals to an all-or-none E2F response. The positive-feedback loops that affect E2F activity (Box 1, Figure I) are, most probably, an essential feature of this bistable switch. Additional studies are required to fully understand the role of specific activator E2Fs and positive-feedback loops in this bistable switch.

E2F and apoptosis

In light of its pro-proliferative function, it seems paradoxical that one member of the E2F family, E2F1, can also mediate apoptosis. Ectopic expression of E2F1 leads to apoptosis in tissue-culture cells and transgenic mice [18]. Notably, E2F1-mediated apoptosis has a physiological role and is not just an artificial result of ectopic expression; mice deficient in E2F1 exhibit an excess of mature T cells owing to a defect in thymocyte apoptosis [19]. Also, E2F1 knockout mice develop tumors, in part, owing to suppressed apoptosis [19].

Other E2Fs, in particular E2F3, have been shown to induce apoptosis in some experimental systems [20–22]. However, E2F3-induced apoptosis is E2F1 dependent and is largely attributed to the ability of E2F3 to transactivate the E2F1 gene [23]. Therefore, although apoptosis induction might not be unique to E2F1, other E2Fs might not trigger apoptosis directly. Loss of either E2F1 or E2F3 inhibits the aberrant apoptosis and proliferation observed in Rb-deficient mice, implicating these E2Fs in pRB-dependent apoptosis [5]. However, an analysis of Rb<sup>−/−</sup> chimeras shows that apoptosis of pRB-deficient cells can be prevented by the presence of neighboring pRB-expressing cells, thereby indicating that pRB suppresses apoptosis in a non-cell-autonomous manner [24]. This finding raises the possibility that, similarly, E2F1 loss suppresses aberrant apoptosis in Rb<sup>−/−</sup> mice in a non-cell-autonomous manner. These data notwithstanding, in mouse-brain epithelium, inactivation of Rb induces p53 activation and apoptosis, both of which are inhibited by E2F1 deficiency in a tissue-autonomous manner [25]. E2F1 induces apoptosis via p53-dependent and p53-independent pathways.
Box 1. Feedback loops in the Rb–E2F pathway

The Rb–E2F pathway has a central role in determining cell proliferation and viability and is, therefore, tightly regulated. One facet of this tight regulation is the complex web of feedback loops that exists within this pathway and between E2F family members (Figure I). E2F activates expression of cyclin E, which, together with CDK2, phosphorylates pRB, further releasing E2F from the inhibitory grip of pRB. Thus, the E2F–cyclin E–pRB interactions constitute a positive-feedback loop. In addition, expression of the genes encoding E2F1, E2F2 and E2F3a is regulated by E2F, generating another positive-feedback loop. Consequently, expression of these three E2Fs is cell-cycle regulated, with a peak of expression at late G1 and early S phase [5]. E2F1 also activates the DNA-damage-induced kinases ATM and Chk2, which, in turn, phosphorylate E2F1, forming a potential third positive feedback loop.

The Rb–E2F pathway also includes a few negative-feedback loops. One mechanism to repress the activity of activator E2Fs involves cyclin A, which is itself an E2F-regulated gene. Upon E2F-mediated expression of cyclin A, the cyclin A–CDK2 complex binds to the E2F1, E2F2 and E2F3a proteins during S phase, leading to the phosphorylation of their dimerization partner (DP) and the inhibition of E2F–DP DNA-binding activity [60]. E2F1 also upregulates the expression of the deacetylase SirT1, which binds to E2F1 and inhibits its activities, constituting a second negative-feedback loop. The physiological relevance of these interactions is highlighted by the increase in the transcriptional and apoptotic activity of E2F1 following SirT1 inactivation [61]. A third possible negative-feedback loop involves Skp2 (S-phase kinase-associated protein 2), an E2F-regulated gene [62] encoding a ubiquitin E3 ligase that targets E2F2 to degradation [63]. However, Skp2 and E2F2 have also been suggested to function in a positive-feedback loop that affects the restriction point and includes cyclin E–CDK2, pRB–E2F2, SKP2 and p27, another pivotal target of SKP2-mediated degradation [64]. In addition, E2Fs regulate the expression of Rb, Arf, p21, p27 and p18INK4C, all of which might function in negative-feedback loops that limit E2F level or transcriptional activity: ARF mediates degradation of activator E2Fs [65], whereas pRB and the CDK inhibitors limit the activity of the activator E2Fs. Feedback loops also exist between E2F and microRNAs (Box 2).

Importantly, E2F1 positively regulates the expression of the repressor E2Fs, E2F7 and E2F8. One of their key targets for transcriptional repression is the E2F1 gene; combined ablation of E2F7 and E2F8 results in an increase in E2F1 levels and activity, leading to apoptosis [8]. It is striking that none of the aforementioned negative-feedback mechanisms are sufficient to effectively restrict E2F1-induced apoptosis in E2F7 and eight double-knockout mice. This indicates that the many negative-feedback loops in the Rb–E2F pathway are, at least to some extent, non-redundant, perhaps because they function at particular conditions or at distinct time frames along the cell cycle. Altogether, the feedback loops enable both the regulation and the fine-tuning of E2F, which is crucial to its proper and timely activity.

![Figure I. Positive- and negative-feedback loops in the Rb-E2F pathway.](image)

**E2F1-induced p53-dependent apoptosis**

The p53 tumor suppressor is a key player in the cellular response to various stresses, including DNA damage and oncogene activation. After such stresses, p53 elicits one of a few possible responses, such as growth arrest or apoptosis.

In some settings, E2F1 signaling to p53 is mediated by Arf, a gene that is transcriptionally activated by E2F2 and encodes a protein that stabilizes and activates p53 [26]. Interestingly, Arf expression is not upregulated by E2F2 during normal cell-cycle progression. Only deregulated-E2F activity leads to Arf expression and p53 activation. However, E2F1 has been shown to induce p53-dependent apoptosis in Arf-deficient mice and cells, indicating that there must be additional, ARF-independent, functional links between E2F1 and p53. Indeed, E2F1 can induce phosphorylation of p53 on residues that are phosphorylated in response to DNA damage and, in this way, trigger activation of p53 [27–29]. Accordingly, E2F1 has been found to influence the expression and activity of the DNA-damage-responsive kinases ATM (ataxia telangiectasia mutated) and Chk2 (checkpoint kinase 2), which phosphorylate and activate p53 [28–30] (Figure 2). Of note, ARF, ATM and Chk2 all have p53-independent activities and, therefore, their activation by E2F1 might also have a role in E2F1-mediated p53-independent apoptosis.

Because p53 is capable of mediating various stress responses, the question arises, how does E2F1-dependent activation of p53 result specifically in apoptosis? In addition to activating p53 itself, E2F1 up-regulates the expression of pro-apoptotic co-factors of p53, such as ASPP (apoptosis stimulating protein of p53) 1 and ASPP2, thereby biasing p53 to activate pro-apoptotic genes and induce apoptosis [31–33]. Furthermore, it has been reported that E2F1 can bind p53 and, thus, stimulate its apoptotic function in response to DNA damage [34]. Notably, this pro-apoptotic interaction between p53 and E2F1 is independent of E2F1 transcriptional activity.

pRB is functionally inactivated in most human tumors, resulting in deregulated and hyperactive E2F. In this context, E2F1-induced apoptosis is viewed as a fail-safe mechanism that suppresses transformation. In support of this premise, Rb is often inactivated together with components of the Arf–Mdm2–p53 pathway; this is thought to reflect selective pressures to reduce the otherwise apoptotic consequences of deregulated-E2F1 activity.

**E2F1-induced p53-independent apoptosis**

E2F1 can also induce apoptosis in the absence of p53. E2F1-induced p53-independent apoptosis is attributed mainly to E2F-mediated up-regulation of various pro-apoptotic genes. The list of such death-inducing E2F1-regulated genes is constantly growing and includes genes encoding Apaf1 (apoptotic protease activating factor 1), caspases, Bcl-2 homology 3 (BH3)-only proteins, and the p53 family member p73 [18]. However, E2F1 mutants lacking the transactivation domain can induce apoptosis efficiently [18], indicating that, at least in some settings, E2F1-dependent gene derepression has a pivotal role in cell death. In addition, it has been found that E2F1 sensitizes cells to apoptosis by inhibiting survival signals, in particular, those mediated by the transcription factor nuclear factor-
κB (NF-κB) or by Bcl-2 and its family member myeloid cell leukemia 1 (Mcl-1) [18] (Figure 2). Thus, E2F1 directs apoptosis via a combination of activation, derepression and repression of gene expression. Furthermore, E2F1 might trigger apoptosis in a transcription-independent manner [35]. The exact set of events underlying E2F1-induced apoptosis in each particular case is most probably determined by the cellular context. Moreover, in many cases in which E2F1 is activated and induces proliferation, its activity does not lead to cell death, indicating that the apoptotic potential of E2F1 is tightly regulated during normal growth. In fact, it is widely believed that, in many cases, the apoptotic activity of E2F1 is an anti-tumorigenic function that is switched on in conjunction with aberrant and deregulated proliferation.

**Regulation of E2F1 apoptotic activity**

Although the pro-apoptotic activity of E2F1 is well established, some studies indicate that E2F1 might also have pro-survival activities. For example, E2F1/−/− keratinocytes exhibit enhanced apoptosis in response to UV-B [36]. Also, in the imaginal wing discs of Drosophila, dE2F1 promotes apoptosis of intervein cells in response to DNA damage, but protects cells within the dorsal–ventral boundary from apoptosis [37]. Thus, it seems that E2F1 can induce or inhibit apoptosis depending on cell type, developmental stage and apoptotic stimulus. Moreover, loss of pRB results in E2F1-dependent apoptosis in some tissues and biological settings, but not in others. Furthermore, when normal cells are stimulated to grow, E2F1 activity is required for initial entry into the cell cycle, but the ability of E2F1 to induce apoptosis is blocked, as mentioned. These observations indicate that E2F1-induced apoptosis is under strict control. In recent years, the regulation of E2F1-induced apoptosis has been the focus of intensive study. The variables determining whether active E2F1 leads to apoptosis are now starting to be identified.

One of the first signals recognized to induce E2F1 apoptotic activity was DNA damage. This induction occurs by several molecular mechanisms that affect the pRB–E2F1 interaction, E2F1 stability and/or the binding of E2F1 to promoters of specific E2F-regulated genes (Figure 1). In general, the domain within pRB that mediates binding to E2Fs encompasses amino acids 379–792 and has been named the pRB pocket. In the case of E2F1, an additional C-terminal domain of pRB also binds the transcription factor [38]. This secondary binding site within pRB inhibits specifically E2F1-induced apoptosis and binding of E2F1 to this site is lost after DNA damage [38]. In particular, DNA-damage-induced acetylation of pRB within this domain results exclusively in E2F1 release, without affecting the binding of pRB to other E2Fs, thereby promoting E2F1-induced apoptosis [39]. Furthermore, in response to DNA damage, E2F1, but not E2F2 or E2F3, is phosphorylated by ATM and Chk2, and these phosphorylations stabilize E2F1 [40,41]. It is not clear whether these two kinases cooperate and whether they regulate E2F1 activity in addition to stabilizing the protein. E2F1 also functions upstream of ATM and Chk2; therefore, DNA damage triggers a positive-feedback loop between E2F1 and these kinases [28–30]. Moreover, like pRB, E2F1 also undergoes specific acetylations after DNA damage and it has been suggested that these modifications bias E2F1 towards pro-apoptotic targets, in particular p73 [42]. Taken together, the current data indicate strongly that DNA damage induces changes in E2F1 and its interacting proteins that direct E2F1 to induce apoptosis (Figure 1). Accordingly, some studies find that, in response to chemotherapeutic drugs that damage DNA, cells lacking E2F1 or carrying an RNAi directed against E2F1 exhibit reduced apoptosis [40,43], although other studies do not detect such a reduction. It seems that, even in response to DNA damage, E2F1-induced apoptosis is context dependent.

A conserved region of E2F1 known as the marked box domain was shown to have unique pro-apoptotic activity that distinguishes E2F1 from other E2Fs [44]. Jab1, originally identified as a specificity factor for c-Jun and JunD transcription factors, interacts specifically with E2F1 via this domain and mediates E2F1-induced apoptosis [45] (Figure 1). Thus, Jab1 represents the first, and so far the only, pro-apoptotic co-factor of E2F1 to be identified. The mechanism by which Jab1 affects E2F1-induced apoptosis remains to be determined. The marked box domains of the other activator E2Fs also mediate protein–protein interactions that affect the ability of E2F2 and E2F3a to transactivate specific target genes [46,47]. It is tempting to speculate that proteins other than Jab1 bind to the marked box domain of E2F1, thereby affecting the subset of promoters that it binds and regulates.

Another effector of E2F1-induced apoptosis is apoptotic inhibitor-5 (Api5), which was identified in a genetic screen in flies as a suppressor of E2F1-induced apoptosis in vivo [48]. Api5 functions downstream of E2F1 and does not inhibit E2F1 transcriptional activity. This E2F–Api5 functional interaction is conserved from flies to humans. Interestingly, Api5 levels are elevated in many human tumors and might enable tumor cells to evade E2F1-induced apoptosis [48].

Recent studies highlight the role of signal-transduction pathways in modulating E2F1-induced apoptosis: the epidermal-growth-factor receptor (EGFR)–Ras–Raf signaling inhibits E2F1-induced apoptosis in vivo during the development of Drosophila imaginal eye discs [49] (Figure 1). In this setting, the Rb–E2F pathway and the EGFR–Ras–Raf pathway most probably converge on Hid, a key regulator of cell death in Drosophila. Hid is transcriptionally upregulated by dE2F1 [37] and is inhibited by a Ras- and MAPK (mitogen-activated protein kinase)–induced phosphorylation. An interesting question concerns whether this functional link between the EGFR–Ras and Rb–E2F pathways is evolutionarily conserved between flies and mammals. Ectopic expression of mutant forms of Ras, and consequent activation of Raf, inhibits apoptosis resulting from a triple knockout of all Rb family members in mice [50], indicating that at least part of this functional link is indeed conserved.

However, it seems to be the PI3K–Akt pathway and not the MAPK pathway that inhibits E2F1-induced apoptosis in tissue-cultured mammalian cells [44]. Akt inhibits E2F1-induced apoptosis indirectly by phosphorylating topoisomerase II-binding protein 1 (TopBP1), which, as
a consequence, binds E2F1 and represses E2F1-induced apoptosis [51]. Additionally, a recent study identified a subset of E2F1 target genes required for E2F1-induced apoptosis that are repressed specifically by PI3K-Akt signaling [52]. In line with this, in breast and ovarian cancer patients, low levels of expression of this subset of E2F1 target genes are associated with poor prognosis, indicating that modulating the balance between E2F1-induced proliferation and apoptosis affects the survival of patients [52]. Interestingly, E2F1 activates Akt in human cells, indicating the existence of an Akt–E2F1 feedback loop by which E2F1 might modulate its own apoptotic activity [53].

Taken together, the aforementioned data concerning the regulation of E2F1-induced apoptosis indicate that, in normal cells, in the absence of DNA damage and in the presence of proper external signals for survival and growth, the apoptotic potential of E2F1 is suppressed (Figure 1). Conversely, when normal cells experience DNA damage or lack of growth and survival signals the apoptotic activity of E2F is unleashed. In transformed cells, E2F is often deregulated and hyperactive owing to defects in the Rb pathway and this might trigger apoptosis. However, E2F-induced apoptosis is inhibited in the transformed cells either by mutations in apoptotic pathways downstream to E2F (for example, a mutation in the p53 pathway is often detected in cells with a defective Rb pathway) or by alterations in pathways regulating the apoptotic activity of E2F (for example, a high PI3K activity).

Of note, the recent discovery that the EGFR–Ras–Raf and PI3K–Akt pathways inhibit E2F1-induced apoptosis has potentially important implications for future cancer therapy. Pharmaceutical attenuation of signaling pathways that inhibit E2F1-induced apoptosis might lead to the selective killing of tumor cells that lack functional Rb but retain operational apoptotic machinery downstream of E2F1. However, the distinct signaling pathways that regulate E2F1-induced apoptosis in specific human tissues and tumors must first be better characterized.

E2F – not only proliferation and apoptosis
Although most literature concerning activator E2Fs relates to roles in the G1–S transition and apoptosis, there are a growing number of reports indicating that they affect additional processes, including mitosis, DNA-damage checkpoints and DNA repair [4]. Additionally, studies in animals and cells lacking distinct E2Fs, or distinct E2F combinations, demonstrate clearly that members of the E2F family have a role in developmental processes and in the differentiation of various tissues [4,5]. Some of these effects are most probably consequences of E2F1-induced proliferation and apoptosis; however, several studies indicate that E2Fs regulate differentiation directly [4].

E2F and signal transduction
Several signaling pathways initiated by mitogenic stimuli ultimately converge to inactivate the function of the Rb family, which leads to E2F activation. This exemplifies the well-documented flow of information from the cell membrane to the nucleus. However, several recent studies show that E2F modulates the activity of signal-transduction pathways via transcriptional regulation of upstream components of such pathways. This indicates that information flows also in the unexpected ‘reverse’ direction, from a nuclear transcription factor, E2F, to upstream signaling pathways [54]. For example, E2F affects positively the MAPK–p38 and PI3K–Akt signaling pathways through transcriptional induction of the apoptosis-signal-regulating kinase 1 (ASK1) and the adaptor protein Gab2, respectively [53,55]. It is likely that such E2F-mediated transcriptional regulation of signaling pathways does not, in isolation, activate these pathways, but serves to sensitize cells, enabling them to respond to sub-optimal stimuli. In support of this notion, activation of the Rb–E2F pathway sensitizes fibroblasts to bFGF (basic fibroblast growth factor), most probably through E2F1-mediated transcriptional upregulation of FGF receptor 1 [56], and ectopic expression of E2F1 sensitizes cells to PDGF (platelet-derived growth factor), most probably by E2F-mediated transcriptional activation of the MEK (MAPK kinase)–ERK (extracellular signal-regulator kinase) pathway [57]. Most experiments demonstrating E2F1-signal-transduction crosstalk were performed in cultured cells using ectopically expressed E2F1. It remains to be determined whether such functional interactions exist in vivo. Cancer cells must acquire the capacity to survive and proliferate in an initially unfavorable environment with limited availability of growth and survival factors. Therefore, if physiological, the effects of E2F on signaling pathways (that control cell survival, cell growth and cell proliferation) might be crucial in conferring a selective advantage on cells that exhibit deregulated-E2F activity.

E2F and autophagy
Although the role of E2F in apoptosis is well characterized, little is known regarding its putative participation in other cell-death pathways. Autophagy is an evolutionarily conserved vesicular-trafficking process that mediates the degradation of cytosolic proteins and organelles and is stimulated rapidly in response to various stresses, such as nutrient or growth factor deprivation. Autophagy is implicated in controlling cell viability; in some settings, functioning as a survival mechanism whereas, in others, promoting cell death. Interestingly, Rb-deficient fetal hepatocytes exhibit non-apoptotic death with autophagic features, implicating deregulated E2F in autophagy [58]. Furthermore, the E2F-regulated BH3-only protein Bnip3 was shown to be required for hypoxia-induced autophagic cell death [58]. Also, activation of E2F1 upregulates the expression of four crucial autophagy genes, LC3, ATG1, ATG5 and DRAM, and enhances basal autophagy [59]. Conversely, reducing endogenous E2F1 expression inhibits DNA-damage-induced autophagy [59]. These recent studies implicate the Rb–E2F pathway and, in particular E2F1, in the control of autophagy (Figure 2). Thus, it is conceivable that autophagy and apoptosis are co-regulated at the transcriptional level. Future studies must address in more detail the relationship between E2F1-induced autophagy and E2F1-induced apoptosis. Of note, the nutrient energy sensor AMP kinase α 2 (AMPKα2), which is an inducer of autophagy, is an E2F1 target gene required for
its apoptotic activity [52]. It would be interesting to elucidate the possible role AMPKε2 has in E2F1-induced autophagy and to understand whether and how this affects E2F1-induced apoptosis. An open question is whether E2F2-induced autophagy results in cell death or cell survival. It seems likely that either outcome is possible depending on the autophagic stimulus and cellular context.

Concluding remarks

Clearly, E2F function and regulation are highly sophisticated and, although a vast amount of knowledge has been accumulated concerning this crucial effector of cell fate, many challenges lie ahead. Our understanding has advanced concerning which signals, in particular DNA damage and activation of signal-transduction pathways, determine whether E2F activity will induce cell proliferation or death. These features of cellular context probably determine whether E2F functions in vivo as an oncogene or a tumor suppressor. Nevertheless, more studies are required to fully comprehend the overall output of the many positive- and negative-feedback loops in the Rb–E2F pathway. Also, the integration of microRNAs into the E2F network, both as regulators and as effectors, awaits further study and might indicate useful therapeutic targets (Box 2). Moreover, as novel functions of E2F emerge, such as regulation of autophagy and signaling, future studies should investigate their effect on the overall outcome of E2F activity both in culture and in vivo. Ultimately, a better understanding of how the complex network of signals to and from E2F determines the overall outcome of E2F activity will pave the way to targeting the E2F pathway in cancer therapy.

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