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Transcription Factors of the E2F Family and DNA Damage Control

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ABSTRACT

The ability to sense and respond to genetic lesions is pivotal to maintain the integrity of the genome. This response is composed of cell cycle checkpoints and DNA repair mechanisms that serve to ensure proper replication of the genome prior to cell division. E2F is a family of mostly heterodimeric transcription factors that can be divided into subgroups with opposing activities. E2F factors are intrinsically tied to proliferation and best known for their ability to regulate the timely expression of genes required for replication and cell cycle progression. However, recent studies suggest that E2F can also regulate transcription of genes involved in other biological processes. These include DNA damage response, DNA repair and apoptosis, mitosis and mitotic checkpoints, and differentiation. E2F activity is regulated in a cell cycle-dependent manner, primarily through its interaction with pocket proteins like the retinoblastoma tumor suppressor protein. Pocket proteins themselves are regulated through reversible phosphorylation by cyclin dependent kinases. Among the E2F proteins, the E2F1 transcription factor is of special interest because of its contrasting behavior under cellular stress conditions, which sets it apart from all other members of the family. E2F1 may act as an oncogene or as a tumor suppressor, probably depending on the genetic background and the level of expression. Upon DNA damage, E2F1 becomes the target of damage-induced kinases, which results in dramatic alteration of its stability, interaction partners, and target genes. Here we review the current understanding of the role of E2F proteins with a focus on the regulation and activity of E2F1 during DNA damage.

Keywords: acetylation, apoptosis, ATM, cell cycle, knock out, oncogene, phosphorylation, proliferation, senescence, tumor suppressor
Abbreviations: **AA**, amino acids; **ARF**, alternate reading frame; **ATM**, Ataxia Telangiectasia Mutated; **ATR**, ATM- and Rad3-Related; **CHK**, checkpoint kinase; **ChIP**, chromatin immuno precipitation; **DSB**, double-strand break; **HAT**, histone acetyl transferase; **HDAC**, histone deacetylase; **MEF**, mouse embryonic fibroblast; **NES**, nuclear export signal; **NLS**, nuclear localization signal; **pRB**, Retinoblastoma-tumor-suppressor protein; **ROS**, reactive oxygen species

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INTRODUCTION

Long-lived multicellular organisms have developed regulatory mechanisms allowing proliferation of their cells when required and repressing unwanted growth of altered or damaged cells. The proliferation of normal somatic cells necessitates the presence of mitogenic stimuli while inhibitory signals that are used to survey the response to these stimuli must be overcome. Tumor formation can result from the breakdown of cellular control mechanisms often caused by context and tissue specific combinations of mutations. Although widely disparate by origin and degree of malignancy, a common property of all cancers is the proliferation beyond the boundaries set by these control mechanisms. The number of mutations required for tumorigenic transformation and the signaling pathways affected by these mutations seems to vary widely between tissues but also species. Nevertheless, six essential alterations in cell physiology have been proposed as the hallmarks of cancer: self-sufficiency in growth signals, insensitivity to growth-inhibitory signals,

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evasion of programmed cell death, limitless replicative potential, sustained angiogenesis, and tissue invasion and metastasis (Hanahan and Weinberg 2000). Each of these new capabilities represents a broken line of defense against the formation of cancer and a step further towards malignancy.

DNA damage is often the initiating event in cellular transformation and can be caused by spontaneous mutations during cellular reproduction, by metabolic processes creating free radicals, and by external causes like UV- or X-rays, or chemical carcinogens. The cell reacts to DNA damage by activating checkpoints. These are biochemical pathways that delay or arrest cell cycle progression in response to DNA damage. A checkpoint comprises the signal, sensors of the signal, mediators, transducers, and effectors. However, there is not an absolute distinction between the components of the checkpoint. The primary cellular response to DNA damage is to repair, but if the damage overwhelms the repair capacity, apoptosis is initiated instead. Following damage, the PI3-kinase-related protein kinases ATM (Ataxia Telangiectasia, mutated) and ATR (ATM- and Rad3-Related) become activated and cooperate with other proteins to initiate the DNA damage response (for a recent review see Su 2006).

Among the most dangerous DNA lesions are double-strand breaks (DSBs) that result in the immediate activation of cell cycle checkpoints, the temporary arrest of the cell cycle, and the activation of repair enzymes. In the case of DSBs these checkpoints are orchestrated primarily by a single protein – ATM, which is the product of a gene that is mutated in the genetic disease ataxia telangiectasia (A-T). A-T belongs to the so called "genomic instability syndromes" and is characterized by cerebellar degeneration, immunodeficiency, genomic instability, and extreme sensitivity to DSB-inducing agents (for a review see Shiloh 2003). A rapid increase of ATM's kinase activity can be observed after DSB induction. More than a dozen targets of ATM have been identified so far. One of the main targets is the tumor suppressor p53 that becomes phosphorylated directly by ATM on Ser15 and indirectly on Ser20 via checkpoint kinase 2 (Chk2) (reviewed in McGowan 2002), which is also a substrate of ATM. Stimulation of ATM activity also results in phosphorylation and stabilization of the transcription factor E2F1 (Lin *et al*. 2001). Oncogenic activation of E2F1 in turn stimulates the expression (Berkovich and Ginsberg 2003) and activity of ATM resulting in a positive feedback (Powers *et al*. 2004).

The E2F family of transcription factors is best known for its role in cell cycle regulation (for a review see (Attwooll *et al*. 2004)). More recent work however suggests that E2F is also involved in the response to DNA damage, in repair and checkpoint activities and differentiation. Moreover, chromatin immunoprecipitation assays combined with high-density oligonucleotide arrays indicated that up to 20% of the human gene promoters are bound by E2F (Bieda *et al*. 2006).

All this points out that transcription factors of the E2F family are among the key players that determine the fate of a cell. Here we discuss what is currently known about E2F proteins with a focus on the response to DNA damage and the role of E2F1.

E2F TRANSCRIPTION FACTORS

E2F is a family of mostly heterodimeric transcription factors where each heterodimer comprises one member of the E2F branch and one member of the DP branch of the family. E2F activity is present in all cell types and is conserved from plants to animals. Eight E2F genes are known in mammals, giving rise to at least 10 E2F proteins. These proteins can be divided into four subgroups based on sequence homology, their function, the mechanisms by which this function becomes activated, and complex formation with DP proteins (summarized in **Fig. 1**). E2F1, E2F2, and E2F3a represent one subgroup and E2F3b, E2F4, and E2F5 the second one. E2F6 is so far the only member of the third

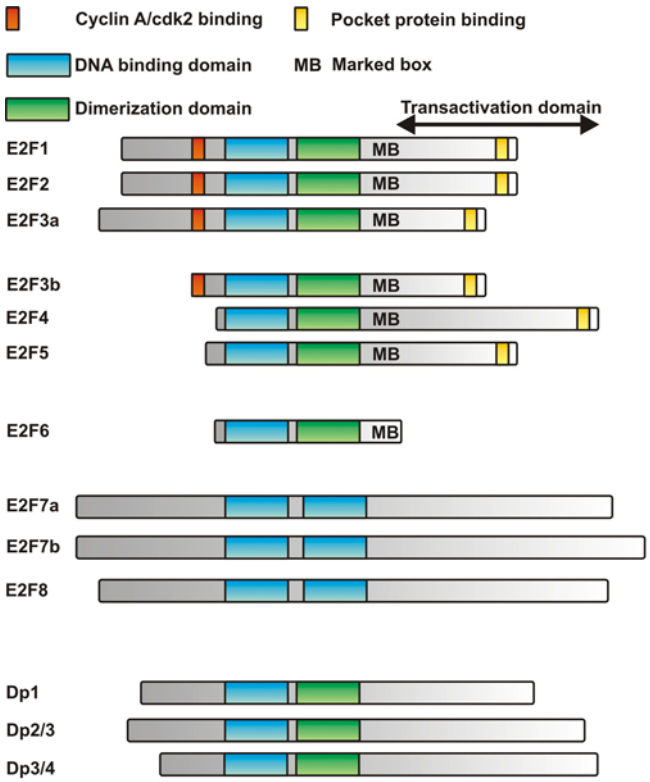

Fig. 1 Structure and functional domains of E2F and DP proteins. E2F proteins can be divided into four classes. E2F1, 2, and 3a are activators, whereas E2F3a, E2F4, and E2F5 are pocket protein-dependent repressors. The third subgroup consists only of E2F6, which lacks the transactivation domain, but carries a repression domain. E2F1 through E2F6 specifically bind DNA as heterodimers together with a DP protein. E2F7a, 7b, and 8, composing the fourth subgroup have no heterodimerization domain, but a second DNA binding domain, allowing them to bind DNA without a DP partner. Human DP2 and 3 correspond to murine DP3 and 4.

subgroup. The fourth subgroup consists of E2F7a, E2F7b and E2F8. Functional and structural similarities can be found within each subgroup but are not always restricted to the respective group. E2F1 through E2F6 specifically bind DNA as heterodimers together with a DP protein. Pocket protein binding of the E2F/DP complexes is mainly determined by the E2F component. *In vitro*, all possible combinations of E2F/DP complexes can exist. Potentially, this allows the formation of a great number of heterodimeric complexes *in vivo*. These proteins also have in common a conserved domain called the marked box. E2F1 to E2F5 carry a C-terminal transactivation domain that comprises a binding site for the retinoblastoma tumor suppressor protein (pRB) or its close relatives p107 and p130, collectively known as pocket proteins. E2F1, E2F2, and E2F3a share an N-terminal domain that comprises a nuclear localization signal (NLS) and binding sites for several proteins that influence their activity. E2F4 and E2F5 carry a nuclear export signal (NES) instead (Gaubatz *et al*. 2001).

E2F6 lacks both a transactivation domain and pocket protein binding site but carries a repression domain that binds Ring1 and YY1 binding protein (RYBP), components of the mammalian polycomb (PcG) complex (reviewed in Trimarchi and Lees 2002).

E2F7a, 7b (de Bruin *et al*. 2003; di Stefano *et al*. 2003), and 8 (Christensen *et al*. 2005; Logan *et al*. 2005; Maiti *et al*. 2005) are set apart from all other E2Fs by their ability to bind DNA without a DP partner. These proteins have two DNA binding domains (DBDs) and lack the heterodimerization domain present in all other E2Fs.

The regulatory activity of the E2F/DP complexes seems to be determined primarily by the E2F subunit with the DP proteins playing a role mainly in DNA binding. DP1, the first of the three characterized DP proteins, is a widespread component of E2F complexes (Girling *et al*. 1993). The in-

consistent naming of the other DP proteins has caused some confusion in the literature. The murine form of DP2 (Zhang and Chellappan 1995) is sometimes called DP3 (Ormondroyd *et al*. 1995) and human DP3 corresponds to murine DP4 (Milton *et al*. 2006a). DP2/3 mRNA is subject to extensive alternative splicing giving rise to at least four protein isoforms with varying properties (Ormondroyd *et al*. 1995). Binding of DP3/4 to an E2F partner results in a complex with reduced DNA binding activity. It therefore acts as a repressor of cell cycle progression (Milton *et al*. 2006a).

REGULATION OF E2F ACTIVITY

Cell cycle regulation of E2F

Since the discovery of E2F (Kovesdi *et al*. 1986), considerable evidence has accumulated suggesting a crucial role for this protein family in the control of gene expression during the cell cycle. E2F1, 2, and 3a are transcriptional activators, interact exclusively with pRb, and are periodically expressed during the cell cycle with maximum levels observed in G1 and early S phase. E2F3b also interacts with pRb, whereas E2F4 can bind all three pocket proteins, and E2F5 interacts with p130. These E2F proteins are constitutively expressed and are classified as repressors. It must be kept in mind however, that the actual activity of a given E2F is defined by its interaction with pocket proteins and other cofactors. Repressor E2Fs can activate transcription when overexpressed, and activating E2Fs might act as repressors in certain circumstances when associated with pRb (for a review see Dimova and Dyson 2005). The activities of E2F1-5/DP complexes are negatively regulated by the binding of pocket proteins to the activation domains of the E2F proteins.

In G0 cells an E2F4/DP heterodimer and, to a minor extent, E2F5/DP bound by the pocket protein p130 seems to constitute the major E2F DNA binding activity (Vairo *et al*. 1995) although one group has reported E2F6/DP1 associated with polycomb group proteins being the major G0 E2F binding activity (Ogawa *et al*. 2002). The pocket protein keeps E2F silent by blocking the activation domain of the respective E2F protein. In addition, pocket proteins can actively repress E2F-dependent transcription by associating with chromatin-modifying repressors like histone deacetylases (HDACs), SWI/SNF complexes, Polycomb proteins, or methyltransferases (reviewed in Frolov and Dyson 2004). The association of E2F4 and E2F5 with a pocket protein is required for their nuclear localization (Allen *et al*. 1997). Intracellular localization in turn at least partially determines the activity of E2F4 and E2F5. Supplying E2F4 with a NLS, thus making its nuclear localization independent of a pocket protein, is sufficient to change it to an activator of transcription (Muller *et al*. 1997). In the G0 and early G1 phase, cdk-associated C- and D-type cyclins interact with pocket proteins via their LXCXE motif followed by cyclin E/cdk2 in middle to late G1 phase. These cyclin/cdk complexes phosphorylate the pocket protein, which presumably induces a conformational change to allow dissociation of E2F factors. The dissociation of the pocket protein seems to render DNA binding of E2F4/5 complexes unstable and these E2Fs relocalize to the cytoplasm directed by their nuclear export signals. Newly synthesized E2F1, E2F2, and E2F3 occupy the free E2F binding sites and this coincides with the induction of E2F-responsive genes (reviewed in Cam and Dynlacht 2003). The role of pRb in the control of E2F-regulated promoters is still under debate. Contradicting results were obtained in chromatin immunoprecipitation (ChIP) experiments investtigating the promoter occupancy of pRb (Morrison *et al*. 2002; Rayman *et al*. 2002; Wells *et al*. 2003). The major pRb associated E2F activity in quiescent cells seems to be the E2F3b/pRb complex that might bind only specific promoters (Leone *et al*. 2000). pRb may therefore be recruited to repress only specific promoters in normal cycling cells and act as a general repressor under some, but not all conditions that result in cell cycle arrest. Particularly in cells undergoing differentiation or senescence, pRb could be involved in transcriptional regulation. ChIP experiments carried out in our laboratory with wild type pRb and a mutant defective for cdk phosphorylation showed, that the mutant but not wt pRb could be readily detected at promoters (Sarr, unpublished results). This suggests that in most cases pRb becomes phosphorylated immediately or shortly after DNA binding of the associated E2F and rapidly dissociates. Why this does not seem to be the case for the E2F3b/pRb complex is currently unclear. Besides pRb, E2F1 also becomes phosphorylated at Ser 332 and 337, which prevents binding of pRb irrespective of its phosphorylation status and increases the stability of E2F1 (Fagan *et al*. 1994). The recruitment of activating E2Fs coincides with the emergence of histone acetyltransferase (HAT) activity, which brings forward acetylation of nucleosomes, resulting in gene activation (Takahashi *et al*. 2000). However, the expression of some E2F regulated genes is delayed and may require the binding of other transcription factors in addition. Interestingly, at some promoters, E2F1-3 binding seems to differ from E2F4 and 5 binding (Araki *et al*. 2003; Zhu *et al*. 2004). To make the situation even more complex, binding to certain promoters seem to require the cooperation of specific E2Fs with other transcription factors (Karlseder *et al*. 1996). Individualized regulation of gene expression may be achieved by this complex pattern of E2F activity.

E2F1, E2F2, and E2F3 comprise a conserved domain within their N-termini that allows stable association with cyclin A/cdk2 during S-phase. This results in phosphorylation of both the E2F and the DP part of the complex with a concomitant loss of DNA binding activity (Dynlacht *et al*. 1994; Krek *et al*. 1994). This phosphorylation is a prerequisite for cell cycle progression. Expression of mutated E2F1 lacking the cyclin A binding site or DP1 lacking the respective phosphorylation sites arrests cells in the S-phase (Krek *et al*. 1995). Pocket protein bound E2F is protected from ubiquitin-dependent degradation by the masking of C-terminal sequences (Hateboer *et al*. 1996; Hofmann *et al*. 1996). In the late S/G2 phase however, E2F1-3, unprotectted by pRb, becomes polyubiquitinated and degraded via the proteasome pathway. Phosphorylation by TFIIH/cdk7 seems to be at least in the case of E2F1 the trigger for this degradation (Vandel and Kouzarides 1999).

Other regulatory mechanisms

E2F activity is controlled not only by pocket proteins that determine the stimulating or repressing function of E2F1 to E2F5 complexes. Further levels of specificity can be obtained by selective DNA binding site recognition, by interaction with particular protein partners, or by posttranslational modifications that alter DNA binding, activity or stability of a given E2F complex. Selective DNA binding site recognition has been found at the Mcl-1 promoter (Croxton *et al*. 2002a). Particularly striking is the inactivation of pocket proteins by the early gene products of the small DNA tumor viruses that results in increased E2F activity and actually lead to the discovery of E2F (Kovesdi *et al*. 1986). Simian virus 40 (SV-40) and murine polyoma virus large T (LT) antigen, adenovirus E1A, and human papilloma virus (HPV) E7 are oncoproteins that bind pRb and other pocket proteins and this results in the release of trancriptionally active E2F complexes (reviewed in Cress and Nevins 1996).

E2F1, E2F2, and E2F3a share an N-terminal domain that comprises a nuclear localization signal (NLS), a cyclin A/cdk binding site, a binding site for transcription factors of the Sp1 family (Rotheneder *et al*. 1999) and a binding site for a protein called EAPP (Novy *et al*. 2005). The role of the E2F/Sp1 interaction seems to be promoter-specific and can either result in synergistic activation (Karlseder *et al*. 1996) or in repression of transcription in G0 and early G1 (Jensen *et al*. 1997). EAPP stimulates the expression of cell cycle regulated genes in an E2F dependent manner. Remarkably, the human $p14^{ARF}$ promoter that is strongly acti-

vated by E2F1 becomes repressed by EAPP (Novy *et al*. 2005). It would be interesting to find out whether EAPP also binds to E2F3b that acts as a specific repressor of the $p14^{ARF}$ gene and might mediate the repressive effect of EAPP.

The levels and the activity of E2F1-3 seem to become reduced by the binding of murine $p19^{ARF}$ (Martelli *et al*. 2001), or human $p14^{ARF}$ (Mason *et al*. 2002) and this appears to favor cell cycle arrest over apoptosis. The interaction with $p19^{ARF}$ stimulates the binding of Skp2, the cell cycle regulated component of the SCF^{SKP2} complex (Marti *et al*. 1999). Moreover, $p19^{ARF}$ directly interacts with DP1 and inhibits its interaction with E2F1 (Datta *et al*. 2005), indicating that ARF influences E2F activity by several mechanisms. Binding of the pleiotropic regulator Purα results in reduced DNA binding and at the same time in increased stability of E2F1. Interestingly, Purα also interacts with pRB and might interfere with the formation of the pRB/E2F1 complex (Darbinian *et al*. 1999). The potential tumor suppressor prohibitin is able to inhibit E2F-dependent transcription by interacting with both, an E2F protein and a pocket protein (Wang *et al*. 1999). This repression presumably requires the recruitment of corepressors (Rastogi *et al*. 2006). IgM stimulation can release prohibitin-mediated repression of E2F activity in B cells and this correlates with the dissociation of both, prohibitin and pRb from E2F1 (Wang *et al*. 1999).

The interferon-inducible protein p202 also reduces E2F-dependent transcription and this may contribute to the growth inhibitory effects of interferon (Choubey *et al*. 1996).

E2F KNOCK OUT MICE

Over-expression of E2F1 is sufficient to induce DNA replication and ectopically expressed E2F1 cooperates with activated ras in transformation assays, demonstrating that E2F1 can be oncogenic. It was expected that the inactivation of the E2F1 gene in the mouse germline would yield underdeveloped or absent tissues, although the multitude of E2F factors suggested some functional redundancy. Instead, the outcome of the first inactivation studies was fully unanticipated (Field *et al*. 1996; Yamasaki *et al*. 1996). Mice lacking E2F1 are viable and fertile but exhibit hyperplasia and even neoplasia during aging. Inactivation of E2F2 (Murga *et al*. 2001) as well as the E2F1/E2F2 (Zhu *et al*. 2001) double knock out results in increased proliferation of hematopoetic cells. E2F1/2 double knock out mice are highly prone to develop cancer and, to a lesser extent, autoimmunity. E2F3 deficient mice arise at only one quarter of the expected frequency, demonstrating that E2F3 is important for normal development (Humbert *et al*. 2000b). Double knockouts of either E2F1/3 or E2F2/3 are embryonic lethal. Conditional ablation of E2F3 from E2F1/2 double knockout mouse embryonic fibroblasts containing a floxed E2F3 allele abolished any proliferation (Wu *et al*. 2001). Surprisingly, this does not seem to result primarily from the loss of activation of E2F target genes, but rather from the recruitment of p53 to its target genes, among them $p21^{CIP1}$. The expression of p21 results in inhibition of cdk activity and subsequently in pocket protein hypophosphorylation. This in turn promotes the formation of pocket protein E2F4 complexes and the repression of E2F target genes. Inactivation of p53 in this setting results in reactivation of E2F target genes and proliferation (Timmers *et al*. 2007). However, there seems to be a difference between acute loss of individual E2Fs and long term loss that allows cells to adapt. Acute loss of either E2F1 or E2F3 blocks S phase entry after stimulation of quiescent cells. A clear distinction of the gene expression pattern can be observed after acute loss of either E2F1 or E2F3 (Kong *et al*. 2007). Long-term loss seems to allow compensation by the upregulation of other family members that take over the function of the lost protein and enable the cell or the organism to survive. E2F4 -/- mice displayed a variety of erythroid abnormalities and died of an increased susceptibility to opportunistic infections, demonstrating that E2F4 is essential for normal development (Humbert *et al*. 2000a; Rempel *et al*. 2000). Development of E2F5 knockout embryos appeared normal, but newborn mice developed non-obstructive hydrocephalus (Lindeman *et al*. 1998). Simultaneous inactivation of E2F4 and E2F5 in mice results in neonatal lethality, suggesting that they perform overlapping functions during mouse development (Gaubatz *et al*. 2000). Mice lacking E2F6 are viable and healthy but display homeotic transformation of the skeleton similar to those observed in polycomb knockout mice (Storre *et al*. 2002). Contrary to the relatively mild phenotype resulting from the inactivation of individual E2Fs, the loss of DP1 leads to death *in utero* (Kohn *et al*. 2003). DP1 is absolutely required for extra-embryonic development and this cannot be rescued by p53 inactivation. The consequences of knock outs of E2F7 and E2F8 as well as of DP2 and DP3 have not yet been reported.

The phenotypes of the knock out mice suggest that E2F family members have unique but overlapping roles *in vivo* and many but not all functions of individual E2Fs can be taken over by their siblings. The reactivation of E2F target genes and thus proliferation in cells having lost E2F1, 2, and 3 (triple knock out MEFs) by p53 deletion indicates, that the main role of E2Fs would be to repress E2F target genes. However, some E2F targets like PCNA are strongly upregulated, while others like Cdc6 are downregulated in these triple knockout MEFs. This suggests that a subset of E2F targets requires the activating function of E2F, whereas others are mainly regulated by repression. Future studies with E2F binding sites mutated in their natural setting might provide definitive answers to this question.

E2F TARGET GENES

Since the discovery of E2F as a cellular DNA-binding activity required for transactivation of the adenovirus E2 promoter by the E1A oncoprotein (therefore E2F = E2 factor) (Kovesdi *et al*. 1986) and the subsequent identification of the first cellular E2F regulated promoter (Blake and Azizkhan 1989) an ever growing number of putative E2F binding sites has been reported. The discovery that E2F is connected with pRb and thus with cancer dramatically increased the efforts to find E2F regulated genes. Genes already known to become induced at the G1/S boundary were the logical first choice in the quest for E2F binding sites. Several E2F-regulated genes that play a role in DNA replication and cell cycle control were identified this way (for a review see Muller and Helin 2000). A special case is the promoter of the $p14^{ARF}$ ($p19^{ARF}$ in mice) gene. It seems to be constitutively bound and repressed by an E2F3b/pRB complex in normal cells. Oncogenic signaling results in the binding of E2F1 and, to a lesser degree, also of E2F2 and E2F3a and the activation of this promoter (Aslanian *et al*. 2004). A computer analysis of promoter databases to search for E2F binding sites suggested that about 7% of a sample of mammalian promoters is regulated by E2F (Kel *et al*. 2001). Examination of expression patterns with DNA microarrays revealed that again about 7% of the studied human mRNAs responded to overexpression of an E2F (Muller *et al*. 2001). The advent of the chromatin immunoprecipitation (ChIP) method allowed studying the *in vivo* association of E2F factors with their corresponding binding sites (Weinmann *et al*. 2001). The combination of microarray and chromatin immunoprecipitation in ChIP on chip experiments lead to the identification of many hundreds of genes that are bound and regulated by E2F proteins (Ren *et al*. 2002; Weinmann *et al*. 2002). These approaches have confirmed that the expression of many genes involved in cell cycle control and DNA replication is regulated by E2F. Some unexpected findings also emerged from these studies. Many of the newly identified E2F targets are not induced in late G1 but rather in early G1 or S/G2. The timely stimulation of the respective promoters might be achieved by the combinatorial action of E2F and other factors. Even more surprising,

E2Fs seem to regulate the expression of genes with functions like DNA damage response, apoptosis and differentiation that are unrelated to cell cycle regulation (reviewed in Bracken *et al*. 2004). Statistical analysis of ChIP on chip data from arrays representing 30 Mb (1% of the human genome) suggested that E2F1 could bind more than 20% of human promoters, frequently at non-canonical sites (Bieda *et al*. 2006). E2F transcription factors may therefore play a pivotal role in the transcriptional regulation of cellular processes far beyond the originally described cell cycle and proliferation.

SPECIFIC PROPERTIES AND FUNCTIONS OF E2F1

E2F1, the first identified and by far the best-studied member of the E2F family, has functions that sets it apart even from its siblings of the activating subset of E2F proteins. Besides its well-examined role in cell cycle regulation it is able to trigger apoptosis as a component of the checkpoint and stress response machinery. And although E2F2 and 3 are also able to induce apoptosis, this depends on the accumulation of E2F1 (Lazzerini Denchi and Helin 2005). This is consistent with the finding that E2F1 but no other E2Fs can act as a tumor suppressor (Yamasaki *et al*. 1996) and that E2F1 is upregulated in response to DNA damage. Whether increased E2F1 activity turns out to be oncogenic (by stimulating proliferation) or tumor suppressive (by inducing apoptosis or senescence) seems to depend on the genetic background of the respective cell or organism (reviewed in Bell and Ryan 2004).

In addition to the well-examined regulation by pRb, E2F1 activity is modulated by a multitude of protein-protein interactions and posttranslational modifications (**Table 1; Fig. 2**). Many of these interactions and modifications occur only after a cell encounters stress like DNA damage or the expression of cellular and viral oncoproteins.

Many signaling pathways induced by cellular stress converge on the p53 tumor suppressor. Activation of p53 results either in growth arrest or apoptosis. Deletion or mutation of p53 predisposes cells to the development of cancer. Alterations in the p53 gene are the most common genetic defects found in tumors so far, around 50% of human tumors carry p53 mutations. Following stress, p53 is covalently modified by phosphorylation at numerous serine and threonine residues and acetylated on lysine residues. Several kinases have been identified that play a role in the activation of p53 after DNA damage. Among them ATM, the kinase mutated in ataxia telangiectasia (AT) and ATR the ATM and Rad3-related kinase. ATM is primarily activated in response to double strand breaks (DSBs), whereas ATR reacts to a wide range of damage including stalled replication forks. ATM and ATR amplify the damage signal by phosphorylating their downstream targets, checkpoint kinase1 (Chk1) and checkpoint kinase2 (Chk2) (reviewed in Ward and Chen 2004).

The observation that treating cells with DNA damaging agents increases E2F1 levels suggested a role for E2F1 in the DNA damage response (Blattner *et al*. 1999; Hofferer *et al*. 1999). The identification of E2F1 as a direct target for phosphorylation by ATM and ATR (Lin *et al*. 2001) and the discovery that E2F1 serves as a crucial link between the pRb and p53 pathways shed new light on the role of E2F1 (Rogoff *et al*. 2002).

Interest in the unique properties of E2F1 has brought forth numerous studies indicating that it plays an important role in checkpoint signaling following DNA damage but also after the activation of oncogenes or the loss of tumor suppressors.

DNA damage specific modifications and interactions of E2F1

It turned out that the increase of E2F1 is caused by the damage induced PI3 related kinases ATM and ATR and their downstream targets Chk2 and Chk1 (Lin *et al*. 2001; Stevens *et al*. 2003). ATM/ATR phosphorylate E2F1 within the binding site for the F-box protein Skp2, on Ser31. Skp2 is the cell cycle regulated component of the ubiquitin ligase SCF^{Skp2} and its binding mediates the proteasome dependent degradation of E2F1 (Marti *et al*. 1999). The ATM/ATR catalyzed phosphorylation of E2F1 seems to interfere with the binding of Skp2 and thus with the degradation of E2F1. However, as binding of the SCF complex is usually induced and not inhibited by phosphorylation, it seems likely that proteins recognizing and binding E2F1 phosphorylated on Ser31 are the true protectors from degradation. And indeed, this phosphorylation not only stabilizes E2F1 but also results in novel protein-protein interactions with diverging consequences for the fate of the cell. Binding of TopBP1 (DNA topoisomerase II beta binding protein I) to E2F1 phosphorylated on Ser31 abolishes both, S phase function and apoptosis induction by inhibiting the transcriptional ac-

Table 1 E2F1 interacting proteins.

Protein	Binding region	Role of interaction	Reference
EAPP	N-terminus	Activation	Novy *et al*. 2005
GABPγ1	Transactivation domain	Activation	Hauck *et al*. 2002
Sp1,2,3,4	AA 102-125	Activation	Rotheneder *et al*. 1999
ASC-2	Transactivation domain	Activation	Kong *et al*. 2003
PARP1	?	Activation	Simbulan-Rosenthal *et al*. 2003
ACTR	AA 1-284	Activation	Louie *et al*. 2004
DDB	AA 363-437	Activation	Hayes *et al*. 1998
DP1	AA 206-220	Stimulation of DNA binding	Krek *et al*. 1993
Prohibitin	AA 284-357	Repression	Wang *et al*. 1999
p53	AA 68-108	Stimulation of apoptosis	Hsieh *et al*. 2002
PURα	?	Repression	Darbinian *et al*. 1999
MDMX	AA 117-241	Repression of DNA binding	Strachan *et al*. 2003
Chk2	?	Phosphorylation and stabilization	Stevens *et al*. 2003
pRB	AA 409-426	Repression	Cao *et al*. 1992
$p14^{ARF}$	AA 181-261 (and AA 426-437)	Repression	Mason *et al*. 2002
p202	AA 88-214	Repression	Choubey *et al*. 1996
TopBP1	N-terminus (Phospho-S31)	Repression, DNA repair?	Liu *et al*. 2004
Cyclin A/cdk2	AA 68-108	Repression of DNA binding, Phosphorylation	Dynlacht *et al*. 1994; Krek *et al*. 1994
14-3-3τ	N-terminus (Phospho-S31)	Stabilization	Wang *et al*. 2004
MDM2	AA 359-407	Stabilization	Martin *et al*. 1995
ATM/ATR	?	Phosphorylation and stabilization	Lin *et al*. 2001
p300/CBP	Transactivation domain	Acetylation and stabilization	Galbiati *et al*. 2005
P/CAF	?	Acetylation and stabilization	Martinez-Balbas *et al*. 2000
Jab1	AA 252-386	Stimulation of apoptosis	Hallstrom and Nevins 2006
SirT1	AA 283-437	Repression of apoptotic activity	Wang *et al*. 2006
pRB	AA 1-374	Repression of apoptotic activity	Dick and Dyson 2003
Api5	?	Repression of apoptotic activity	Morris *et al*. 2006
Skp2	N-terminus	Ubiquitination	Marti *et al*. 1999
TFIIH	AA 409-437	Phosphorylation, degradation	Vandel and Kouzarides 1999
NBS1	AA 284-416	Checkpoint activity	Maser *et al*. 2001

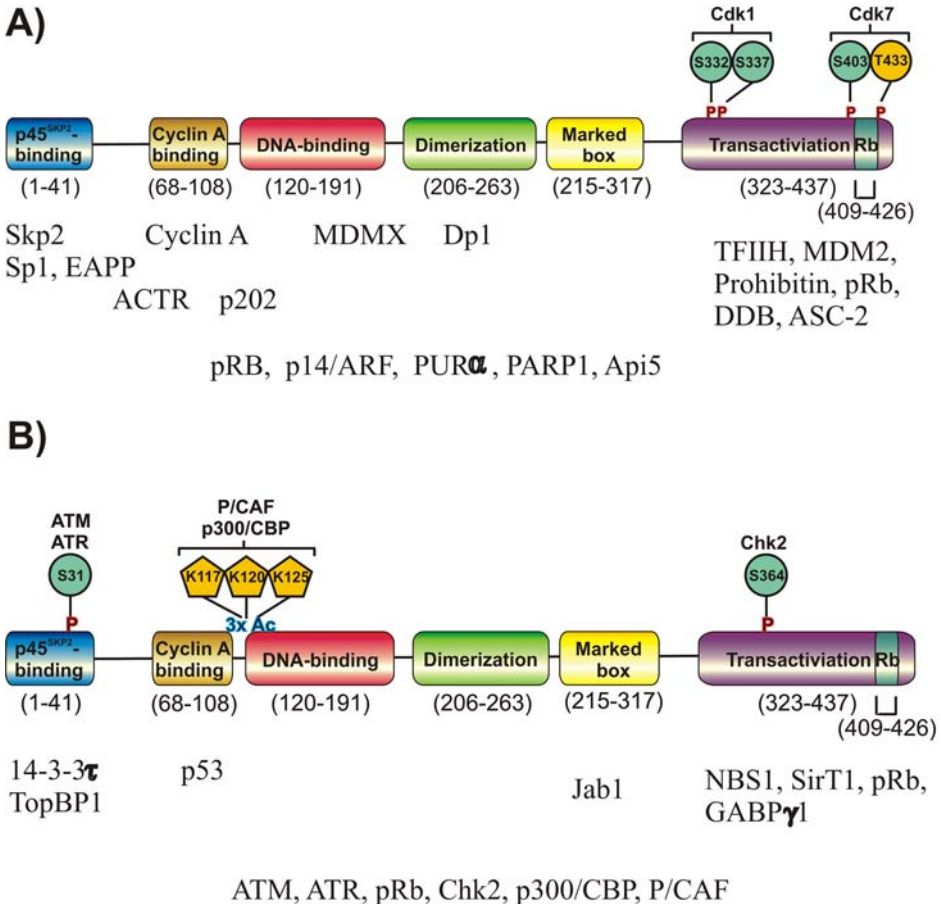

Fig. 2 Schematic representation of the domains of E2F1, their posttranslational modifications, and interacting proteins. (**A**) Cell cycle-dependent modifications and interactions, (**B**) DNA damage-induced modifications and interactions

tivity of E2F1. This seems to be achieved by recruiting Brg1/Brm, an important component of the SWI/SNF chromatin-remodeling complex (Liu *et al*. 2004). The interaction with E2F1 also requires oligomerization of TopBP1 and this depends on phosphorylation by Akt (Liu *et al*. 2006). Moreover, the TopBP1/E2F1 complex relocalizes to stalled replication forks together with BRCA1, Nbs1, and 53BP1, suggesting a role for E2F1 in DNA repair activities (Liu *et al*. 2003). This is in line with a report showing an interaction of E2F1 with the Mre11 complex (comprising Mre11, Rad50, and Nbs1) via Nbs1, which seems to suppress the firing of origins of replication upon DNA damage (Maser *et al*. 2001).

A weak interaction of the protein 14-3-3τ with E2F1 is strongly enhanced following DNA damage and phosphorylation on Ser31 of E2F1. This interaction interferes with the degradation of E2F1, as overexpression of 14-3-3τ inhibits, and depletion of 14-3-3τ increases ubiquitination of E2F1. Moreover, 14-3-3τ is required for E2F1-dependent expression of proapoptotic proteins like p73, Apaf1, and caspases (Wang *et al*. 2004). Interestingly, the interaction of 14-3-3ε, another member of the 14-3-3 family, with the NLS containing isoforms of murine DP3 (DP2 in humans) is also under DNA damage control. Contrary to the effects seen with the E2F1/14-3-3τ complex, DNA damage results in the release of DP3 from the DP3/14-3-3ε complex and this induces apoptosis (Milton *et al*. 2006b).

Sequence comparison of the phosphorylation sites of known Chk2 target sites led to the identification of a putative phosphorylation site at Ser364 within the C-terminus of E2F1. Phosphorylation of this site following DNA damage was confirmed and it was shown that the expression of a dominant negative Chk2 mutant blocks E2F1-dependent apoptosis (Stevens *et al*. 2003). Chk1 and Chk2 seem to stimulate the expression of p73 after DNA damage in an E2F1-dependent manner (Urist *et al*. 2004). It will be of interest to determine how the phosphorylation of Ser364 regulates the stability and activity of E2F1. Phosphorylation is not the only DNA damage associated modification of E2F1. Acetylation on lysines 117, 120, and 125 of E2F1 as well as on the corresponding lysines of E2F2 and E2F3 have been reported. Whereas one group (Marzio *et al*. 2000) assigned this acetylation to the acetyl-transferases p300 and CBP, another group ascribed it mainly to the p300/CBP-associated factor P/CAF and only to a lesser extent to p300/CBP itself (Martinez-Balbas *et al*. 2000). Moreover, it was suggested that acetylation increases the half-life of E2F1, and pRb bound HDAC can deacetylate E2F1. Subsequent studies confirmed the acetylation and showed that it strongly increases following DNA damage independently of ATM-mediated phosphorylation of E2F1 (Ianari *et al*. 2004; Galbiati *et al*. 2005). However, the discrepancy concerning the roles of p300/CBP and P/CAF remains, although it was suggested that this reflects the differing regulation of cell cycle regulated and stress-induced E2F target genes (Pediconi *et al*. 2003). According to this model, P/CAF-mediated acetylation of E2F1 results in the redirection of E2F1 to promoters activated during DNA damage. This is supported by the observation that the E2F1(K/Q) mutant that mimics acetylation of lysines 117, 121, and 125 has a high apoptotic activity and becomes efficiently recruited to the p73 promoter (Pediconi *et al*. 2003).

E2F1 and apoptosis

Ectopic expression of E2F1 can drive quiescent cells into S phase indicating that it has a role in proliferation. However, in the absence of growth factors, E2F1 can also induce apoptosis (Qin *et al*. 1994; Wu and Levine 1994). This ability is unique among E2F proteins (Kowalik *et al*. 1998) and also shown *in vivo* by E2F1 knock out mice that exhibit defects in apoptosis (Yamasaki *et al*. 1996). Originally, the apoptotic activity of E2F1 was thought to be similar to the activity of the oncoprotein c-Myc. Elevation of c-Myc levels results in a potent proliferation stimulus and can be found in many tumors (Dang *et al*. 1999). However, without appropriate survival signals the deregulation of c-Myc leads to programmed cell death (Evan *et al*. 1992). This is not al-

ways the case for E2F1 as mutants have been described that retain the ability to cause apoptosis while unable to promote proliferation (Phillips *et al*. 1997). The ability to promote both, cell cycle progression and cell death seems to be safeguard mechanism for the case that DNA damage occurs during DNA synthesis. Several pathways seem to exist how E2F1 can induce apoptosis and the specific role of E2F1 seems to depend on the kind of stress that results in E2F1 induction. E2F1 activated by DNA damage seems to provoke apoptosis via different pathways than E2F1 induced by oncogenic stress (Pediconi *et al*. 2003; Powers *et al*. 2004).

Moreover, distinct mechanisms seem to exist that require different functions and functional domains of E2F1. These include: A) Mechanisms that require the full activity as a transcription factor including the transactivation domain of E2F1. These mechanisms may or may not depend on p53 (see below). B) At least for some mechanisms the transactivation domain seems to be dispensable but they require DNA binding activity (Hsieh *et al*. 1997; Phillips *et al*. 1997). In these cases the binding of an activation deficient E2F1 might repress anti-apoptotic genes (Croxton *et al*. 2002b) or de-repress the expression of apoptosis stimulating proteins (Hershko and Ginsberg 2004). C) A very recent paper reports the surprising finding that a peptide consisting only of the 75 amino acids of the E2F1 DNA binding domain that can neither heterodimerize with DP proteins, nor bind DNA, is capable of efficiently inducing apoptosis (Bell *et al*. 2006). On the other hand it was demonstrated, that the marked-box domain of E2F1 is absolutely required for the induction of apoptosis (Hallstrom and Nevins 2003). More recently, the same group identified Jab1 as an E2F1 specific binding protein and could show that the interaction of the two proteins is required to drive cells into apoptosis (Hallstrom and Nevins 2006). The marked box has been shown to confer promoter specificity to E2F2 and E2F3 (Schlisio *et al*. 2002). Whether this is also the case for E2F1 and Jab1 remains to be investigated. Binding of the ETS related transcription factor GABPγ1 to the C-terminal region of E2F1 increases the proliferation stimulating transcriptional activity and suppresses E2F1 mediated apoptosis. This seems to be at least partly achieved by the repression of pro-apoptotic gene transcription (Hauck *et al*. 2002). E2F1 specific binding of pRb has been reported that differs from the binding to the transactivation domain found in E2F1 to 4 and that is inhibiting E2F1 driven apoptosis. This requires a large N-terminal part of E2F1 (amino acids 1-374) and the resulting complex has low affinity for DNA. Upon DNA damage changes take place that inhibit this specific interaction (Dick and Dyson 2003). These changes might be damage specific acetylation of pRb on lysines 873/874, which results in the release of E2F1 specifically bound by the C-terminal domain of pRb (Markham *et al*. 2006). This acetylation retains pRb in the hypophosphorylated state and allows the pocket-specific binding of E2F2 and 3. Modifications of E2F1 like phosphorylation by ATM and Chk2 (see below) might then inhibit the association of E2F1 with the pocket domain of pRb. The increased apoptosis seen in pRb knock out mouse embryos that can be rescued by the concomitant loss of E2F1 (Tsai *et al*. 1998) could result from this specific interaction. Api5 (apoptosis inhibitor 5), a protein upregulated in cancer cells functions as a strong inhibitor of E2F1-dependent apoptosis (Morris *et al*. 2006). Further, MDM2 was shown to bind to E2F1 (Martin *et al*. 1995) and inhibit E2F1 induced apoptosis (Loughran and La Thangue 2000). The binding of MDM2 prolongs the half-life of E2F1 by displacing Skp2 (Zhang *et al*. 2005) and thus inhibiting degradation of E2F1. Unclear is however, why the resulting higher E2F1 levels do not give rise to apoptosis. Possibly apoptosis-specific modifications of E2F1 (see above) are also prevented by MDM2.

Other experiments have indicated that E2F1 can also act as an inhibitor of apoptosis in response to UVB radiation (Wikonkal *et al*. 2003). Whether these sometimes contradictory findings can be ascribed to differing experimental settings or can be explained by yet unknown regulatory mechanisms remains to be seen.

p53-dependent induction of apoptosis by E2F1

E2F1 is able to procure apoptosis via several pathways either dependent or independent of p53. The p53 dependent mechanisms can comprise the activation of the tumor suppressor $p14^{ARF}$. The 1β promoter of the human $p14^{ARF}$ gene is strongly activated by E2F1 (Bates *et al*. 1998). $p14^{ARF}$ has been shown to bind to the p53/MDM2 complex and prevent p53 degradation (Stott *et al*. 1998) by sequestering MDM2 into the nucleolus (Weber *et al*. 1999). A novel E2F-binding element has been identified within the $p14^{ARF}$ promoter that responds to ectopic E2F1 expression and RNAi mediated inactivation of pRb but not to phosphorylation of pRb by serum stimulation (Komori *et al*. 2005). This might allow a cell to distinguish abnormal growth signals and normal cell cycle regulated pRb inactivation. In p53 deficient cells, induction of $p14^{ARF}$ seems to be able to induce apoptosis via Bax upregulation (Li *et al*. 2006). However, the actual role of $p14^{ARF}$ in E2F1 mediated DNA damage response has been questioned by the observation that *in vivo* binding of E2F1 is rather decreased than increased following DNA damage (Pediconi *et al*. 2003). This is in line with a more prominent role of $p14^{ARF}$ as a sensor of oncogenic stress and inducer of senescence.

p53-dependent but $p14^{ARF}$-independent apoptosis can also be induced by E2F1 (Russell *et al*. 2002). At least in part this seems to be the consequence of an upregulation of ATM. E2F1 stimulates ATM promoter activity resulting in an increase of both, ATM mRNA and protein levels. This is accompanied by increased p53 phosphorylation, indicating that ATM also becomes activated (Berkovich *et al*. 2003). The finding that E2F2 is also able to activate ATM, which results in increased p53 phosphorylation but not in apoptosis led to the conclusion that E2F1-induced apoptosis requires additional effectors. It turned out, that the expression of Chk2 is upregulated by E2F1 but not by E2F2 (Rogoff *et al*. 2004). In addition, E2F1 induces phosphorylation of Chk2 at Thr68 in an ATM- and Nbs1-dependent manner (Powers *et al*. 2004) followed by phosphorylation of p53 at Ser20. As coexpression of Chk2 permits E2F2 to induce apoptosis and leads to Ser 20 phosphorylation of p53, the critical step seem to be the E2F1 specific increase in Chk2 expression and activity (Rogoff *et al*. 2004). How E2F1 activates ATM in the first place is currently unclear. It has been suggested that pRb inactivation and the concomitant deregulation of E2F1 blocks the repair of spontaneously occurring damage, leading to the accumulation of DNA double strand breaks (Pickering and Kowalik 2006). However, nonclassical DNA damage independent mechanisms of ATM activation have also been suggested (Hong *et al*. 2006). p53 activation can either result in cell cycle arrest or apoptosis. E2F1 seems to shift the balance in favor of apoptosis. E2F1 might achieve this on the one hand by stimulating the expression of four proapoptotic cofactors of p53 – ASPP1, ASPP2, JMY and TP53INP1 (Hershko *et al*. 2005) and on the other hand by increasing the levels of caspases (see below). In addition, a direct interaction of E2F1 with p53 via the cyclin A binding domain of E2F1 has been reported. In response to DNA damage, this interaction seems to enhance the apoptotic activity of p53 (Hsieh *et al*. 2002).

p53-independent induction of apoptosis by E2F1

The p53 homologue p73 has been identified as a major p53-independent mediator of E2F1 induced apoptosis. The p73 promoter harbors several E2F binding sites and is strongly induced by E2F1 (Irwin *et al*. 2000). DNA damage results in increased binding of E2F1 and a reduction of promoter bound E2F4 (Pediconi *et al*. 2003). The apoptosis protease-activating factor 1 (Apaf1) is a transcriptional target for both, E2F1 and p53 (Moroni *et al*. 2001). Apaf1 is an essential activator of caspases and is required for oncogene-

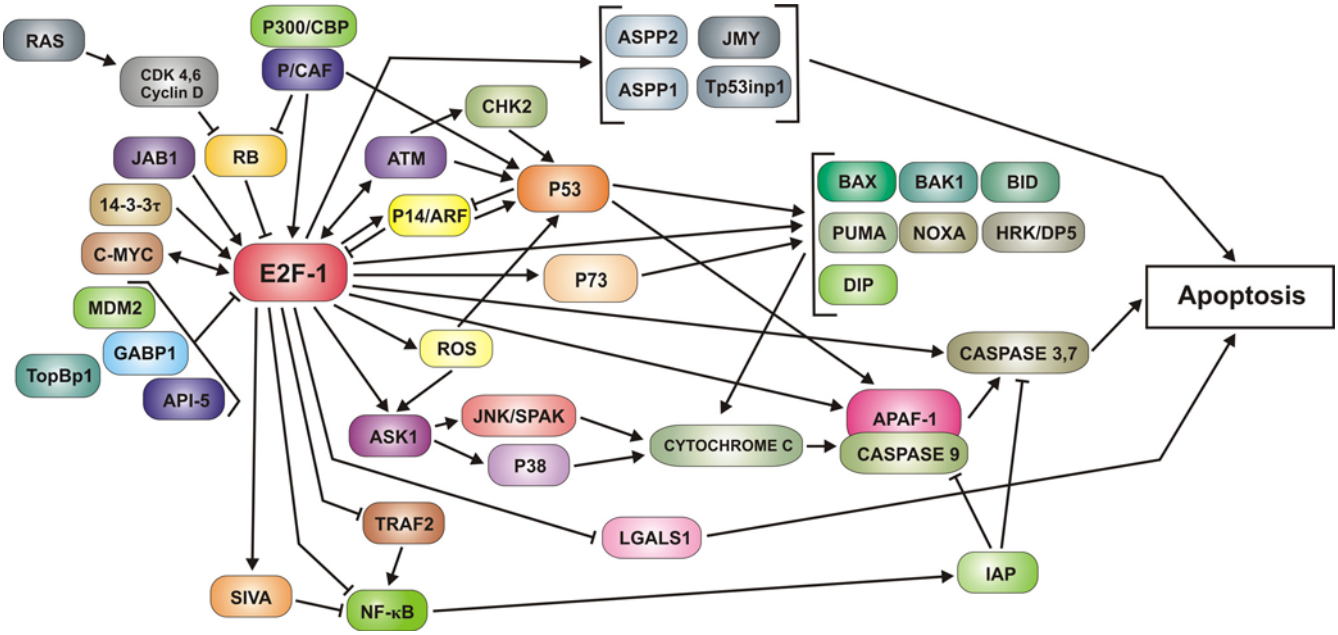

Fig. 3 Role of E2F1 in stress-response and apoptosis pathways. Following stress like DNA damage, the function of E2F1 changes from a stimulator of proliferation into an inducer of apoptosis or senescence. It becomes the target of modifying enzymes like the kinase ATM, or the acetyl transferases p300/CBP or P/CAF. These modifications result in altered protein-protein interactions, protein stability, and DNA binding activity, specifying the course of action of E2F1 during stress. This includes an enhanced interaction with proteins stimulating the apoptotic activity of E2F1 like Jab1 and 14-3-3τ but might also result in reduced binding of apoptosis inhibitors like Api5, GABP or of pRb specifically bound to the N-terminus of E2F1. The interaction with $p14^{ARF}$ seems to determine whether the stress-induced activation of E2F1 results in apoptosis or senescence. Stress-primed E2F1 exerts its activity mainly via the transcriptional activation of a great number of proteins involved in apoptosis. These can be other transcription factors like p53 or p73, proapoptotic components of signal transduction pathways like the ASPP proteins and the Bcl2 homology 3 (BH3)-only proteins PUMA, NOXA, BIM, or proteins directly involved in the execution of apoptosis like the caspases 3 and 7 and Apaf1. In some rare cases, E2F1 can also act as a repressor of antiapoptotic proteins like NFκB. But E2F1 can also enhance apoptosis by stimulating the accumulation of reactive oxygen species (ROS).

induced apoptosis, which is impaired in Apaf1 defective cells. ChIP assays demonstrated that E2F1 binds to the Apaf1 promoter after overexpression (mimicking oncogenic activation) (Furukawa *et al*. 2002) but not after DNA damage (Pediconi *et al*. 2003). Loss of pRb activity or overexpression of E2F1 results in elevated levels of caspases. Reporter assays and ChIP experiments demonstrated that E2F1 binds to and stimulates the caspase 7 promoter. The presence of E2F binding sites and the coordinate regulation of caspases 2, 3, 7, 8, and 9 by E2F1 indicates that all these caspases are direct targets of E2F1 (Nahle *et al*. 2002). Although not sufficient by itself to trigger apoptosis, high levels of caspases increase the probability that a death-inducing signal overcomes the brakes that normally inhibit easy onset of apoptosis. The expression of the proapoptotic Bcl2 homology 3 (BH3)-only proteins PUMA, NOXA, BIM, and HRK/DP5 is also regulated by E2F1. BH3-only proteins integrate apoptotic stimuli into a common pathway and trigger cell death. Inhibition of their expression diminishes E2F1-dependent apoptosis. Conversely, restraining the expression of E2F1 blocks the upregulation of these BH3-only proteins after DNA damage (Hershko *et al*. 2004). BAD and BAK1 are antagonists of the anti-apoptotic protein Bcl2 and have also been identified as E2F1 targets in microarray and RT-PCR experiments. In the same study a protein termed DIP (death inducing protein) that localizes to mitochondria emerged as an E2F1 target. DIP induces cell death without p53 and partially independent of caspases (Stanelle *et al*. 2004). SIVA is a proapoptotic protein that interacts with the tumor necrosis factor receptor family and contains a death domain. Treatment with DNA damaging substances results in the stimulation of the SIVA promoter by both, p53 and E2F1 (Fortin *et al*. 2004).

Activation of the apoptotic pathway mediated by death receptors occurs as a consequence of TNFα (tumor necrosis factor α) binding. The receptors recruit procaspases, the subsequent cleavage of which results in apoptosis. This outcome is not inevitable, since the concomitant activation of NFκB can inhibit apoptosis. Expression of E2F1 prevents the induction of NFκB and thus promotes apoptosis. This requires the DNA binding activity of E2F1 but not its transactivation domain, as a C-terminally truncated E2F1(1-374), but not a DNA binding mutant (E132) also show this effect. E2F1 impedes the activation of NFκB by blocking the I-κB kinase activity, which is required for the degradation of I-κB and the subsequent translocation of NFκB into the nucleus (Phillips *et al*. 1999). Interestingly, NFκB can have a proapoptotic role in p53 induced cell death (Ryan *et al*. 2000). The pathways and interactions involving E2F1 and resulting in apoptosis are summarized in **Fig. 3**.

E2F1 and metabolism

The deacetylase SirT1 has become famous for promoting lifespan extension during caloric restriction. The activity of SirT1 is NAD-dependent and therefore linked to the metabolic state of the cell. Its expression is stimulated by E2F1 and SirT1 interacts with E2F1. This inhibits E2F1 activities, primarily the apoptotic functions, thereby forming a negative feedback loop. The regulation seems to occur by a combination of E2F1 deacetylation and promoter targeting (Wang *et al*. 2006).

Interestingly, another NAD-dependent enzyme, PARP1 (poly-ADP-ribose-polymerase 1) that catalyzes poly-ADP-ribosylation of nuclear substrates acts as a coactivator of E2F1 during reentry of quiescent cells into the S phase (Simbulan-Rosenthal *et al*. 2003). An interaction of GSK3β (Glycogen Synthase Kinase 3β) that is involved in glycogen metabolism with the transactivation domain of E2F1 has also been described (García-Alvarez *et al*. 2007). This interaction is independent of GSK3β kinase activity and results in reduced transcriptional activity of E2F1. The interaction of these proteins with E2F1 might provide a link between the metabolic status, cell cycle regulation, and stress response in a cell.

E2F1-induced senescence

When normal somatic cells undergo replicative senescence, they irreversibly lose the ability to proliferate. Senescent

cells can survive for long periods of time and exhibit an altered spectrum of expressed genes. Telomere shortening during replication resulting in one or more critically short telomeres is in most cases the physiological inducer of senescence. However, in normal human cells, DNA damage or oncogenic stress can also promote senescence, contrary to immortal cells, which rather undergo apoptosis (for a review see Cristofalo *et al*. (2004)).

E2F1 is among the genes that are repressed in senescent cells. Interestingly, ChIP experiments revealed the presence of pRB on E2F regulated promoters in senescent cells. This is in contrast to growing or quiescent cells were promoter bound pRB has rarely been detected (Narita *et al*. 2003). Moreover, senescence specific corepressors are recruited by the antiproliferative protein prohibitin (Rastogi *et al*. 2006). Overexpression of E2F1 induces a senescence-like phenotype in normal human fibroblasts that is associated with a flat morphology, growth arrest and the expression of SA-β-GAL, a senescence-specific marker (Rastogi *et al*. 2006). One consequence of E2F1 overexpression is a dramatic increase of $p14^{ARF}$ expression, comparable to the levels seen in replicatively senescent cells. This in turn results in elevated p53 levels and both, $p14^{ARF}$ and p53 are required for the induction of the senescent phenotype (Dimri *et al*. 2000). Increasing p53 levels by $p14^{ARF}$ mediated inactivation of MDM2 might not suffice, as ATM activity also seems to be needed to initiate senescence (Mallette *et al*. 2007). ATM expression and activity are also stimulated by E2F1 (Berkovich *et al*. 2003) and this leads to p53 phosphorylation and activation. The necessity of several critical tumor suppressors for the initiation of senescence may explain why this pathway is usually inactive in immortal and transformed cells. How the E2F1 triggered senescent phenotype is maintained is currently unclear but likely requires at least in part the same mechanisms as replicative senescence (Cristofalo *et al*. 2004).

CONCLUSIONS

Within the E2F family, E2F1 is unique in exhibiting activity that can result either in cell cycle progression, apoptosis, or senescence. Considering normal cell cycle progression, there is evidence that E2F1 is mainly required for the first cycle out of quiescence, whereas E2F3 is needed to enter the S phase during each cycle. Depending on the level of expression and the genetic background of a cell, E2F1 may function either as an oncogene or as a tumor suppressor. In many tumors the mechanisms that link E2F1 to apoptosis have been interrupted, for example through the inactivation of p53. Induction of apoptosis by E2F1 apparently occurs via different pathways. Some of them necessitate the ability of E2F1 to stimulate the expression of target genes, for others DNA binding and thus repression of target promoters is sufficient. Even the expression of a deletion mutant comprising only the DNA binding domain, which is not able to bind DNA any more, seems to be sufficient to cause apoptosis. It was an intriguing discovery that E2F1 is not only the target of DNA damage induced kinases, but can itself activate these kinases and thus the DNA damage response pathways. But elevated levels of E2F1 activity can also result in senescence. Moreover, the interaction with proteins involved in metabolic processes suggests, that E2F1 also might serve as a node that integrates metabolism with cell cycle regulation and apoptosis. All this indicates that E2F1 is a significant part of the tumor surveillance mechanisms of the cell.

Several questions concerning the role of E2F1 remain. What determines the course of action of E2F1 during DNA damage response? Are higher levels of E2F1 *per se* sufficeent to induce the expression of genes required for programmed cell death? Or is the activation of the DNA damage response pathway with the subsequent post-translational modifications and modification-induced interactions of E2F1 a necessary precondition for E2F1-triggered apoptosis? Is any target gene selectivity mediated by these damage-specific modifications and interactions? There are indications that this is indeed the case, e.g. the recruitment of acetylated E2F1 to the p73 promoter. An important decision maker for the course of E2F1 activity seems to be the ARF protein. Virtually all human tumors have lost the activity of either p53, ARF, or both, indicative of a synergistic role of these proteins. ARF expression is strongly induced by E2F1 and this in turn results in elevated p53 levels and in the right genetic background this leads to senescence. But ARF is also a negative regulator of E2F1 and high ARF levels favor senescence whereas cells having lost ARF seem to be more likely to undergo apoptosis. The biggest remaining question might concern the role of E2F in the development of human cancer. The pivotal role of E2F proteins in balancing the pathways leading to cell cycle progression, arrest, senescence, or apoptosis implies that alteration of their activity may often contribute to tumorigenesis. No unequivocal model has emerged yet that can integrate all the observed activities and effects of E2F proteins and particularly of E2F1. Some findings are quite contrary to much of what we consider consolidated knowledge about E2F. This could mean that there is no universally valid answer to the question of E2F function but may depend on context like genetic background, tissue, and metabolic state of a cell or even the age and immune status of an organism. There can be no doubt that future research will yield new insights and might even result in new therapeutic agents that target E2F proteins and take advantage of their various activities.

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