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INVITED REVIEW

E2F transcription factor action, regulation and possible role in human cancer

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Abstract. E2F transcription factors regulate expression of a panel of cellular genes that control cellular DNA synthesis and proliferation, either by activating or repressing their transcription, largely in a cell cycle-dependent manner. The ability of E2F proteins to regulate expression of these target genes is, in turn, regulated by other cellular proteins that are important for normal control of cell cycle progression. Together, E2F proteins, their target genes, and the proteins that regulate E2F activity comprise a genetic pathway that is probably the most frequently altered pathway in human cancer. This review examines this genetic pathway and focuses on the role of E2F proteins in its function. Specifically, the target genes regulated by E2F, the likely mechanisms by which activation and repression of target gene transcription is achieved, and the regulation of E2F activity by other proteins in the cell, are discussed.

From one perspective, human cancer is a diverse group of diseases affecting many different cell and tissue types. From another, the genetic perspective, cancer is more homogeneous since defects in identical genetic pathways can cause development of diverse cancer types. The appeal of a small number of genetic pathways being responsible for a majority of human cancers clearly is that diagnostic and therapeutic efforts can be focused in a single direction, possibly on only a small number of genes.

One excellent set of candidates for genes participating in many human cancers are those encoding the E2F family of transcription factors (see Farnham 1996 for a collection of reviews on E2F). The E2F gene family contains five known members (Slansky & Farnham 1996), designated E2F-1 through E2F-5. At the molecular level, E2F proteins heterodimerize with proteins encoded by another gene family, called DP (La Thangue 1994). Together, the E2F/DP protein complexes bind to DNA and regulate transcription of a panel of target genes in the cell. E2F proteins have been shown to be rate-limiting regulators of cell cycle progression (Johnson *et al.* 1993, Dobrowolski *et al.* 1994, Sala *et al.* 1994, Suda *et al.* 1994,

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Lukas *et al*. 1996, Shan, Durfee & Lee 1996) and presumably fulfill this role by regulating transcription of target genes.

Even though abnormal E2F activity has not yet been conclusively demonstrated in any human cancer, it is likely that such activity contributes to human malignancy. Why is this? Within the past few years, E2F transcription factors have emerged as central components of possibly the most frequently affected genetic pathway in human cancer (Sherr 1996). Many of the genes that comprise this pathway function to regulate E2F activity and alterations in these regulators of E2F are found in a majority of human malignancies. Since the genes that make up this pathway converge on and exert their effects through E2F transcription factors, it is likely that cancers containing genetic alterations in this pathway result from altered E2F activity and, consequently, altered expression of E2F target genes.

This review will summarize the current status of E2F transcription factors as rate-limiting regulators of cellular proliferation. Specifically, the review will discuss the target genes regulated by E2F, the molecular mechanisms by which target gene regulation is achieved, and the ways in which E2F activity itself is regulated. The emphasis will be on E2F proteins as central regulators of a genetic pathway that is frequently associated with human cancer.

TARGET GENES REGULATED BY E2F

As transcription factors, E2F proteins regulate gene expression by binding to specific DNA sequences within promoter elements of target genes. E2F binds to DNA with the consensus 5′-TTTSSCGC-3′ (S = C or G) sequence (see Slansky & Farnham 1996) and multiple cellular genes contain this sequence in their promoters.

What can be said about the target genes of E2F transcription factors? As effectors of the E2F genetic pathway and possible mediators of malignancy, they might be expected to function as rate-limiting regulators of cellular proliferation. To a large extent, the proteins encoded by these genes fulfill that role. E2F target genes include cellular oncogenes, tumour-suppressor genes, and other rate-limiting regulators of DNA synthesis and cell cycle progression (Table 1). Given the functions of these genes, it is reasonable to expect that their altered expression could contribute to the development of cancer.

Not only do E2F transcription factors regulate the levels of target gene expression, but they do so largely in a cell cycle-dependent fashion. For the most part, E2F proteins ensure that target genes are maximally transcribed in the late G_1 and early S phases of the cell cycle (see Slansky & Farnham 1996). Abnormal expression of E2F could alter this pattern of target gene expression and conceivably contribute to carcinogenesis, not only by changing the overall expression levels of target genes, but by changing the cell cycle-dependent expression pattern of these genes as well.

MECHANISMS BY WHICH E2F REGULATES TARGET GENE TRANSCRIPTION

Experiments have been done in which E2F binding sites in the promoters of target genes were mutated. These experiments suggest that E2F proteins can act both as activators and repressors of target gene transcription. Deletion of E2F binding sites in the c-*myc* promoter, for example, resulted in reduced transcription from these genes (Thalmeier *et al*. 1989, Moberg *et al*. 1992), suggesting that E2F proteins are transcriptional activators in this context. Mutations in E2F binding sites in the B-*myb* promoter, however, caused increased

transcription from this gene (Lam & Watson 1993), suggesting that E2F proteins act as transcriptional repressors here. What determines whether a specific target gene will be activated or repressed by E2F is not entirely clear. Recent experiments, however, provide some insights.

Activation of target gene transcription by E2F (Figure 1A) appears to occur as follows. In early-to-mid G_1 phase of the cell cycle, as well as in non-cycling G_0 cells, E2F proteins are bound by proteins of the retinoblastoma (Rb) tumour-suppressor gene family (i.e. pRb, p107, or p130). Binding of E2F proteins by retinoblastoma proteins (pRb) prevents target gene activation (Figure 1A). To activate target genes, therefore, E2F must be released from binding by pRb. How does this happen?

As cells approach the late G_1 phase of the cell cycle, pRb-family proteins are phosphorylated by cellular kinases that are complexes between cyclins and cyclin-dependent kinases (CDKs). Cyclin D/CDK4, cyclin D/CDK6 and cyclin E/CDK2 phosphorylate pRb proteins (Weinberg 1995). Phosphorylation causes pRb proteins to release bound E2F. Whereas E2F that is bound by pRb cannot activate target genes, unbound or 'free' E2F can stimulate target gene transcription by binding to target gene promoters (Figure 1A).

In mid S phase of the cell cycle, after E2F has activated target gene transcription, E2F activity is inhibited. This occurs when E2F/DP complexes are themselves inactivated by phosphorylation (Krek *et al.* 1994). In addition, as cells progress through M phase and into the next cell cycle, pRb loses phosphorylation (Buchkovich, Duffy & Harlow 1989, Chen *et*

Table 1. Partial listing of E2F target genes*

Functional category of target gene	Specific genes
DNA synthesis/nucleotide metabolism	Carbamoyl-phosphate synthase–aspartate Carbamoyltransferase–dihydroorotase
	DNA polymerase α
	Deoxycytosine kinase
	Dihydrofolate reductase (DHFR)
	Proliferating cell nuclear antigen (PCNA)
	Thymidine kinase
	Thymidylate synthetase
	Topoisomerase I
	Ribonucleotide reductase subunit M2
Cell cycle progression	*cdc2*
	Cyclin A
	Cyclin D1
	Cyclin E
Proto-oncogenes	*erb-B*
	Insulin-like growth factor I (IGF-1)
	B-*myb*
	c-*myb*
	c-*myc*
	N-*myc*
Tumour-suppressor genes	Rb
	p107
Others	E2F-1

*The genes in this table were studied either in human, mouse or rat (Farnham, Slansky & Kollmar 1993, DeGregori, Kowalik & Nevins 1995, Slansky & Farnham 1996, Hurford Jr. *et al.* 1997).

al. 1989, DeCaprio *et al*. 1989, Mihara *et al*. 1989, Ludlow *et al*. 1990) and again binds and inactivates E2F.

Although Figure 1A shows the E2F/pRb complex as not binding to DNA in the early-to-mid G_1 phase, complex formation does not preclude interaction of E2F with DNA. In fact, binding of E2F/pRb complexes to some target gene promoters, beginning in late M phase through mid G_1, actively represses transcription (Figure 1B; Weintraub *et al*. 1995, Zwicker *et al*. 1996). Here E2F acts as a tether to attach pRb proteins to DNA. pRb, now in close proximity to DNA, apparently blocks activation of target gene transcription by other required protein factors and may inhibit the ability of E2F to interact with basal transcription factors (Hagemeier, Cook & Kouzarides 1993, Weintraub *et al*. 1995). As in the model for target gene activation, pRb proteins are phosphorylated by cyclin/CDK kinases as cells approach the late G_1 phase and pRb is consequently released from the E2F/pRb complex. In the absence of the pRb tether, target gene transcription is no longer repressed (Figure 1B).

Could transcription of the same target gene be both repressed, by E2F/pRb binding in early G_1, and activated, by free E2F in late G_1 and early S? This probably happens (Wells *et al*. 1997). However, it is also likely that some target genes are exclusively activated while other targets are exclusively repressed by E2F.

What determines whether a gene will be activated or repressed by E2F? Recent experiments suggest key structural differences between the promoters of target genes that are activated and those that are repressed by E2F (Zwicker & Müller 1997). In the mouse B-*myb* gene, a gene known to be repressed by E2F, a DNA sequence located downstream (or 3′) of

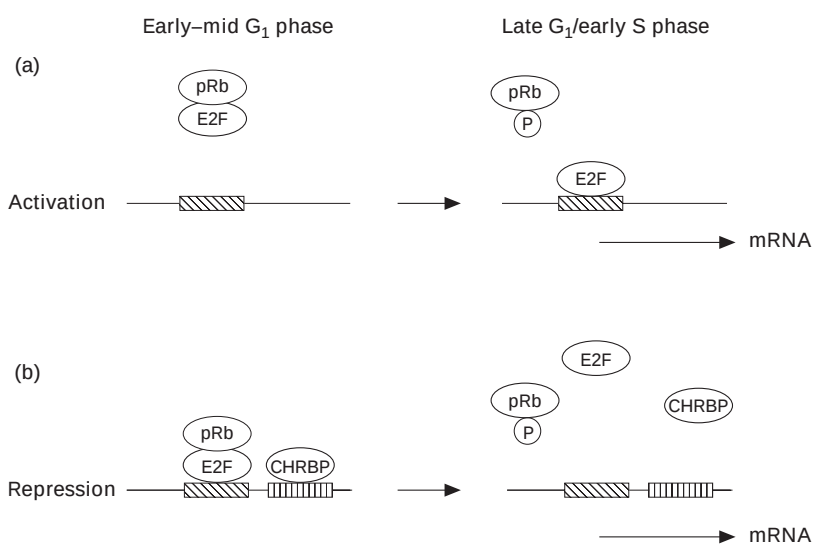

Figure 1. Models for E2F activation and repression of target gene transcription. E2F activation (a) and repression (b) of target gene transcription is shown for both the early-to-mid G_1 and the late G_1/early S phases of the cell cycle and is described in the text. E2F, pRb and CHR binding proteins (CHRBP) are shown interacting with the DNA in the promoter region of a target gene. Binding sites on the DNA for E2F (▨) and CHRBP (▥) are shown. The 'P' attached to pRb represents pRb that has been phosphorylated by cyclin/CDK kinases. The thick lines with arrows at the right ends represent mRNA transcribed from the target gene.

the E2F binding site, called 'cell cycle genes homology region' (CHR), was found (Figure 1B). This CHR element, in combination with an unknown cellular protein (designated CHRBP in Figure 1B) that binds to this sequence, was shown to be necessary for repression of B-*myb* transcription by E2F (Liu & Lucibello 1996).

Therefore, it may be that transcription of target genes containing an E2F binding site, but not a CHR element, are activated by E2F (Figure 1A). Presence of the CHR element downstream of an E2F binding site, plus a CHR binding protein, may determine that target genes will be repressed (Figure 1B).

In addition to the mouse B-*myb* gene, the CHR sequence has been found in the promoters of both human and mouse E2F-1, two genes also known to be repressed by E2F (see Zwicker & Müller 1997). This suggests that these genes may be repressed by a similar mechanism. CHR elements may also play a role in cell cycle specific regulation of cyclin A and *cdc2* gene transcription (Zwicker *et al.* 1995), genes that also contain E2F binding sites in their promoters.

Note that, independent of whether a target gene is activated or repressed by E2F, the transcription pattern will normally be similar (Figure 1). Transcriptional activation of targets by free E2F occurs largely in the late G_1 and early S phases of the cell cycle. Repression by E2F/pRb complexes occurs in cell cycle phases other than late G_1 and early S. Repressed genes normally become transcriptionally derepressed beginning in late G_1.

Despite the fact that the expression patterns are similar, the particular mechanism by which E2F regulates a target should determine the circumstances under which that target gene can contribute to malignancy. For example, overexpression of E2F would increase transcription of target genes activated by E2F (due to increased free E2F) and likely decrease transcription of targets repressed by E2F (due to increased levels of E2F/pRB complexes). Oncogenes that are activated by free E2F and tumour-suppressor genes that are repressed by E2F/pRb complexes could contribute to malignancy under these conditions. Conversely, loss of E2F would decrease transcription of target genes normally activated by E2F (due to decreased free E2F) and increase transcription of targets repressed by E2F (due to decreased levels of E2F/pRb complexes). Tumour suppressors that are activated by E2F, as well as oncogenes that are repressed by E2F could contribute to malignancy when E2F activity is lost.

REGULATION OF E2F ACTIVITY: THE E2F GENETIC PATHWAY

Because they bind to and directly regulate the ability of E2F proteins to affect target gene transcription, pRb-family proteins are immediate regulators of E2F activity. Other cellular proteins regulate the ability of pRb to bind E2F. Still other proteins regulate the proteins that regulate pRb. All of these proteins comprise a linear genetic pathway (Figure 2) that ultimately regulates E2F activity. Genetic alterations in most of the genes shown in this pathway are known to correlate with human malignancy. In fact, the pathway shown in Figure 2 is likely the most commonly affected genetic pathway in human cancer (Sherr 1996). E2F proteins appear to be key transcriptional effectors of this pathway.

In which human cancers do alterations in the genes shown in Figure 2 occur and how might these alterations affect E2F activity? Let us examine this question. First, mutations in the Rb member of the retinoblastoma gene family are common and are frequently found in retinoblastomas and small cell lung carcinomas. Rb mutations are also found in non-small cell lung carcinomas, bladder, breast and prostate carcinomas, leukaemias, malignant gliomas, and many sarcomas (Paggi *et al.* 1996). Mutations that inactivate pRb would be

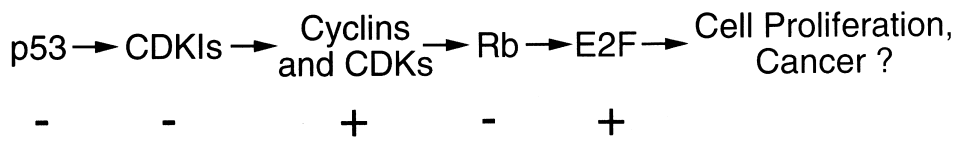

Figure 2. The E2F genetic pathway. The figure illustrates the pivotal location of E2F proteins, downstream of proteins that are essential for cell cycle progression, and upstream of target genes (Table 1) necessary for cell proliferation. Interactions between components of this pathway are discussed in the text. The + and – signs indicate the effects of the indicated component on cell proliferation, the last component in the pathway. For example, increased cyclin or CDK activity would positively affect cell proliferation.

expected to result in increased transcription of target genes that are activated by E2F (due to increased levels of free E2F) as well as target genes that are repressed by E2F (due to decreased levels of E2F/pRb complexes).

Immediately upstream of the Rb gene in the pathway (Figure 2) are the cyclin and CDK genes. Alterations in these genes are also common in human cancers. Amplification of the cyclin D1 gene is frequently associated with head and neck squamous cell carcinomas, oesophageal carcinomas, bladder cancers, ductal breast cancers and hepatocellular carcinomas, and less frequently associated with various other cancers (Hall & Peters 1996). Amplification of the CDK4 gene is common in human sarcomas and gliomas (Hall & Peters 1996).

Since cyclin/CDK kinases phosphorylate pRb-family proteins and, therefore, determine whether pRb will bind E2F, amplification of either the cyclin D1 or CDK4 genes would be expected to increase phosphorylation of pRb proteins, thus decreasing their ability to bind E2F family members. As a result, transcription of target genes activated by E2F (due to increased levels of free E2F) as well as target genes repressed by E2F (due to decreased levels of E2F/pRb complexes) would be expected.

Upstream of cyclins and CDKs in the pathway (Figure 2) are proteins called CDK inhibitors (CDKI). These proteins function to directly inhibit cyclin/CDK activity and, therefore, result in decreased phosphorylation of pRb proteins. p16 is a CDKI that binds and inhibits CDKs that heterodimerize with D-type cyclins (i.e. CDK4 and CDK6) and phosphorylate pRb. Mutations in the p16 CDKI gene are probably the second most common mutation in human cancer (estimated to occur in 20% to 40% of human cancers; Harlow 1996), next to mutations in the gene encoding the p53 tumour-suppressor protein (Sherr 1996). p16 mutations are frequently found in leukaemias, anaplastic astrocytomas, mesotheliomas, biliary tract cancers, nasopharyngeal carcinomas, pancreatic carcinomas, oesophageal carcinomas, bladder and ovarian carcinomas, and non-small cell lung cancers (Hall & Peters 1996).

Loss of CDKI activity would be expected to increase cyclin/CDK kinase activity, thus increasing pRb phosphorylation and decreasing E2F binding by pRb. As a result, free E2F would be expected to increase as would transcription of target genes activated by free E2F. Levels of E2F/pRb complexes would also likely decrease, resulting in increased transcription of target genes that are normally repressed by E2F.

Another important CDKI, p21, can inactivate CDK2 (a partner of cyclin E in pRb phosphorylation), CDK4, CDK6 and others (Harper *et al.* 1995). Mutations in the p21 CDKI gene have not been described in human cancer (Hall & Peters 1996). However, the p53 tumour-suppressor protein is an activator of p21 transcription (Figure 2) and p53 is mutated

in over 50% of human cancers (Hollstein *et al.* 1991, Harris & Hollstein 1993). In these cancers, transcription of the p21 gene should decrease and, since p21 functions to decrease cyclin/CDK kinase activity, p53 mutations would result in increased activity of these kinases. The expected result would be increased pRb phosphorylation and, therefore, increased levels of free E2F. Transcription of target genes activated by E2F would be increased, as would transcription of target genes repressed by E2F (due to decreased levels of E2F/pRb complexes).

Finally, although the interactions between components of the pathway shown in Figure 2 are well established, there are additional interactions, particularly involving pRb proteins, that should be mentioned. pRb proteins appear to bind a number of other cellular transcription factors, in addition to E2F, and regulates the activity of some of them (Sánchez & Dynlacht 1996, Taya 1997). Interestingly, the binding of some of these transcription factors is, like E2F, also regulated by phosphorylation of pRb (Taya 1997). Given these facts, it would not be surprising if some of the other transcription factors bound by pRb also play a role in regulation of cell proliferation and human cancer.

ADDITIONAL DATA IMPLICATING E2F IN HUMAN CANCER

Other evidence suggests that E2F proteins are potentially oncogenic. For example, overexpression of E2F has been shown to cause phenotypic transformation of cultured cells (Johnson *et al.* 1994, Singh, Wong & Hong 1994, Xu, Livingston & Krek 1995, Yang & Sladek 1995). Transformation in these experiments is dependent on the ability of E2F to bind DNA and regulate target gene transcription. E2F mutants that do not retain these properties do not transform (Singh *et al.* 1994, Xu *et al.* 1995).

Similar to some other oncogenes, E2F-1 is also able to cause some cultured cells to undergo apoptosis. Although the original experiments suggested that E2F-mediated apoptosis was dependent on the presence of wild-type p53 tumour-suppressor protein in cells (Qin *et al.* 1994, Wu & Levine 1994, Kowalik *et al.* 1995), some cells containing wild-type p53 do not undergo apoptosis but instead are transformed by E2F-1 overexpression (Sladek 1996). These data might suggest, therefore, that any contribution E2F makes to human cancer could occur in the presence of wild-type p53.

Additional evidence implicating E2F genes in cancer comes from experiments with mutant mice that lack functional E2F-1 (Field *et al.* 1996, Yamasaki *et al.* 1996). Such mice have an increased incidence of tumours. One interpretation of these data is that, in the absence of E2F, genes that are normally repressed by E2F would become transcriptionally active (Weinberg 1996). Given that many E2F target genes regulate cell proliferation (Table 1), derepression of some of these could contribute to tumour formation.

Finally, there are two other reports correlating abnormalities in E2F genes or gene expression with human cancer. In the first report, amplification and overexpression of the E2F-1 gene was found in human HEL erythroleukaemia cells (Saito *et al.* 1995). In the second report, alterations in the E2F-4 gene were detected in colorectal cancers (Yoshitaka *et al.* 1996).

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REFERENCES

BUCHKOVICH K, DUFFY LA, HARLOW E. (1989) The retioblastoma protein is phosphorylated during specific phases of the cell cycle. *Cell* **58**, 1097.

CHEN P-L, SCULLY P, SHEW J-Y, WANG JYJ, LEE W-H. (1989) Phosphorylation of the retinoblastoma gene product is modulated during the cell cycle and cellular differentiation. *Cell* **58**, 1193.

DECAPRIO JA, LUDLOW JW, LYNCH D *et al.* (1989) The product of the retinoblastoma susceptibility gene has properties of a cell cycle regulatory element. *Cell* **58**, 1085.

DEGREGORI J, KOWALIK T, NEVINS JR. (1985) Cellular targets for activation by the E2F1 transcription factor include DNA synthesis- and G_1/S-regulatory genes. *Mol. Cell. Biol.* **15**, 4215.

DOBROWOLSKI SF, STACEY DW, HARTER ML, STINE JT, HIEBERT SW. (1994) An E2F dominant negative mutant blocks E1A induced cell cycle progression. *Oncogene* **9**, 2605.

FARNHAM PJ, ed. (1996) Transcriptional control of cell growth: the E2F gene family. *Curr. Top. Microbiol. Immunol.* **208**, 1–137.

FARNHAM PJ, SLANSKY JE, KOLLMAR R. (1993) The role of E2F in the mammalian cell cycle. *Biochim. Biophys. Acta* **1155**, 125.

FIELD SJ, TSAI F-Y, KUO F *et al.* (1996) E2F-1 functions in mice to promote apoptosis and suppress proliferation. *Cell* **85**, 549.

HAGEMEIER C, COOK A, KOUZARIDES T. (1993) The retinoblastoma protein binds E2F residues required for activation *in vivo* and TBp binding *in vitro*. *Nucleic Acids Res.* **21**, 4998.

HALL M, PETERS G. (1996) Genetic alterations of cyclins, cyclin-dependent kinases, and cdk inhibitors in human cancer. *Adv. Cancer Res.* **68**, 67.

HARLOW E. (1996) A research shortcut from a common cold virus to human cancer. *Cancer* **78**, 558.

HARPER JW, ELLEDGE SJ, KEYOMARSI K *et al.* (1995) Inhibition of cyclin-dependent kinases by p21. *Mol. Biol. Cell* **6**, 387.

HARRIS CC, HOLLSTEIN M. (1993) Clinical implications of the p53 tumor-suppressor gene. *N. Engl. J. Med.* **329**, 1318.

HOLLSTEIN M, SIDRANSKY D, VOGELSTEIN B, HARRIS CC. (1991) p53 mutations in human cancers. *Science* **253**, 49.

HURFORD JR. RK, COBRINIK D, LEE M-H, DYSON N. (1997) pRB and p107/p130 are required for the regulated expression of different sets of E2F responsive genes. *Genes Dev.* **11**, 1447.

JOHNSON DG, CRESS WG, JAKOI L, NEVINS JR. (1994) Oncogenic capacity of the E2F1 gene. *Proc. Natl. Acad. Sci. USA* **91**, 12 823.

JOHNSON DG, SCHWARZ JK, CRESS WD, NEVINS JR. (1993) Expression of transcription factor E2F1 induces quiescent cells to enter S phase. *Nature* **365**, 349.

KOWALIK TF, DEGREGORI J, SCHWARZ JK, NEVINS JR. (1995) E2F1 overexpression in quiescent fibroblasts leads to induction of cellular DNA synthesis and apoptosis. *Mol. Cell Biol.* **69**, 2491.

KREK W, EWEN ME, SHIRODKAR S, ARANY Z, KAELIN WG, JR., LIVINGSTON DM. (1994) Negative regulation of the growth-promoting transcription factor E2F-1 by a stably bound cyclin A-dependent protein kinase. *Cell* **78**, 161.

LA THANGUE NB. (1994) DRTF-1/E2F: an expanding family of hetero-dimeric transcription factors implicated in cell cycle control. *Trends Biochem. Sci.* **19**, 108.

LAM EW-F, WATSON RJ. (1993) An E2F-binding site mediates cell-cycle regulated repression of mouse B-myb transcription. *EMBO J.* **12**, 2705.

LIU N, LUCIBELLO FC. (1996) Cell cycle-regulated repression of B-myb transcription: cooperation of an E2F site with a contiguous corepressor element. *Nucleic Acids Res.* **24**, 2905.

LUDLOW JW, SHON J, PIPAS JM, LIVINGSTON DM, DECAPRIO JA. (1990) The retinoblastoma susceptibility gene product undergoes cell cycle-dependent dephosphorylation and binding to and release from SV40 large T. *Cell* **60**, 387.

LUKAS J, PETERSEN BO, HOLM K, BARTEK J, HELIN K. (1996) Deregulated expression of E2F family members induces S-phase entry and overcomes p16INK4A-mediated growth suppression. *Mol. Cell Biol.* **16**, 1047.

MIHARA K, CAO XR, YEN A *et al.* (1989) Cell cycle dependent regulation of phosphorylation of the human retinoblastoma gene product. *Science* **246**, 1300.

MOBERG KH, LOGAN TJ, TYNDALL WA, HALL DJ. (1992) Three distinct elements within the murine c-myc promoter are required for transcription. *Oncogene* **7**, 411.
PAGGI MG, BALDI A, BONETTO F, GIORDANO A. (1996) Retinoblastoma protein family in cell cycle and cancer: a review. *J. Cell. Biochem*. **62**, 418.
QIN X-Q, LIVINGSTON DM, KAELIN, JR. WG, ADAMS PD. (1994) Deregulated transcription factor E2F-1 expression leads to S-phase entry and p53-mediated apoptosis. *Proc. Natl. Acad. Sci. USA* **91**, 10 918.
SAITO M, HELIN K, VALENTINE MB *et al*. (1995) Amplification of the *E2F1* transcription factor gene in the HEL erythroleukemia cell line. *Genomics* **25**, 130.
SALA A, NICOLAIDES NC, ENGELHARD A *et al*. (1994) Correlation between E2F-1 requirement in the S phase and E2F-1 transactivation of cell cycle-related genes in human cells. *Cancer Res*. **54**, 1402.
SÁNCHEZ I, DYNLACHT BD. (1996) Transcriptional control of the cell cycle. *Curr. Opin. Cell Biol*. **8**, 318.
SHAN B, DURFEE T, LEE WH. (1996) Disruption of RB/E2F-1 interaction by single point mutations in E2F-1 enhances S-phase entry and apoptosis. *Proc. Natl. Acad. Sci. USA* **93**, 679.
SHERR CJ. (1996) Cancer cell cycles. *Science* **274**, 1672.
SINGH P, WONG SH, HONG W. (1994) Overexpression of E2F-1 in rat embryo fibroblasts leads to neoplastic transformation. *EMBO J*. **13**, 3329.
SLADEK TL. (1996) Established cell lines transformed by E2F-1 overexpression contain wild-type p53. *Cell Prolif*. **29**, 579.
SLANSKY JE, FARNHAM PJ. (1996) Introduction to the E2F family: protein structure and gene regulation. *Curr. Top. Microbiol. Immunol*. **208**, 1.
SUDA K, HOLMBERG EG, NORNES HO, NEUMAN T. (1994) DNA synthesis is induced in adult neurons after expression of E2F1 and E1A. *Neuroreport* **5**, 1749.
TAYA Y. (1997) RB kinases and RB-binding proteins: new points of view. *Trends Biochem. Sci*. **22**, 14.
THALMEIER K, SYNOVZIK H, MERTZ R, WINNACKER E-L, LIPP M. (1989) Nuclear factor E2F mediates basic transcription and trans-activation of E1a of the human MYC promoter. *Genes Dev*. **3**, 527.
WEINBERG RA. (1995) The retinoblastoma protein and cell cycle control. *Cell* **81**, 323.
WEINBERG RA. (1996) E2F and cell proliferation: a world turned upside down. *Cell* **85**, 457.
WEINTRAUB SJ, CHOW KNB, LUO RX, ZHANG SH, HE S, DEAN DC. (1995) Mechanism of active transcriptional repression by the retinoblastoma protein. *Nature* **375**, 812.
WELLS JM, ILLENYE S, MAGAE J, WU CL, HEINTZ NH. (1997) Accumulation of E2F-4.DP-1 DNA binding complexes correlates with induction of dhfr gene expression during the G1 to S phase transition. *J. Biol. Chem*. **272**, 4483.
WU X, LEVINE AJ. (1994) p53 and E2F-1 cooperate to mediate apoptosis. *Proc. Natl. Acad. Sci. USA* **91**, 3602.
XU G, LIVINGSTON DM, KREK W. (1995) Multiple members of the E2F transcription factor family are the products of oncogenes. *Proc. Natl. Acad. Sci. USA* **92**, 1357.
YAMASAKI L, JACKS T, BRONSON R, GOILLOT E, HARLOW E, DYSON NJ. (1996) Tumor induction and tissue atrophy in mice lacking E2F-1. *Cell* **85**, 537.
YANG XH, SLADEK TL. (1995) Overexpression of the E2F-1 transcription factor gene mediates cell transformation. *Gene Expr*. **4**, 195.
YOSHITAKA T, MATSUBARA N, IKEDA M *et al*. (1996) Mutations of E2F-4 trinucleotide repeats in colorectal cancer with microsatellite instability. *Biochem. Biophys. Res. Commun*. **227**, 553.
ZWICKER J, LUI N, ENGELAND K, LUCIBELLO FC, MÜLLER R. (1996) Cell cycle regulation of E2F site occupation *in vivo*. *Science* **271**, 1595.
ZWICKER J, LUCIBELLO FC, WOLFRAIM LA *et al*. (1995) Cell cycle regulation of the *cyclin A*, *cdc25C* and *cdc2* genes is based on a common mechansim of transcriptional repression. *EMBO J*. **14**, 4514.
ZWICKER J, MÜLLER R. (1997) Cell-cycle regulation of gene expression by transcriptional repression. *Trends Genet*. **13**, 3.