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MYC AND THE CELL CYCLE

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1. ABSTRACT

Ectopic expression of the c-Myc oncoprotein prevents cell cycle arrest in response to growth-inhibitory signals, differentiation stimuli, or mitogen withdrawal. Moreover, Myc activation in quiescent cells is sufficient to induce cell cycle entry in the absence of growth factors. Thus, Myc transduces a potent mitogenic stimulus but, concomitantly, induces apoptosis in the absence of survival factors. We review here recent progress in our understanding of the molecular mechanisms linking Myc activity to cell cycle control. Myc is a positive regulator of G1-specific cyclin-dependent kinases (CDKs) and, in particular, of cyclin E/CDK2 complexes. Cyclin D/CDK4 and CDK6 may conceivably also be activated by Myc, but the circumstances in which this occurs remain to be explored. Myc acts via at least three distinct pathways which can enhance CDK function: (1) functional inactivation of the CDK inhibitor $p27^{Kip1}$ and probably also of $p21^{Cip1}$ and $p57^{Kip2}$, (2) induction of the CDK-activating phosphatase Cdc25A and (3) - in an ill understood and most likely indirect way - deregulation of cyclin E expression. Constitutive expression of either Myc or cyclin E can prevent growth arrest by $p16^{INK4a}$ (an inhibitor of cyclin D/CDK4, but not of cyclin E/CDK2). In cells, $p16^{INK4a}$ inhibits phosphorylation, and thus induces activation of the Retinoblastoma-family proteins (pRb, p107 and p130). Surprisingly, this effect of p16 is not altered in the presence of Myc or cyclin E. Thus, Myc and cyclin E/CDK2 activity unlink activation of p16 and pRb from growth arrest. Finally, Myc may itself be a functional target of cyclin D/CDK4 through its direct interaction with p107. We discuss how the effects of Myc on cell cycle control may relate to its oncogenic activity, and in particular to its ability to cooperate with activated Ras oncoproteins.

2. INTRODUCTION

2.1. The Myc protein

The *myc* family of proto-*Oncogene*s includes three evolutionarily conserved genes c-, N- and L-*myc*, which encode related proteins. Oncogenic activation of myc genes generally results in their constitutive expression, and contributes to progression of a wide range of human and animal neoplasias (reviewed in 1-3). *myc* genes are differentially expressed during embryonic development (e.g. 4). With few exceptions, proliferating post-natal tissues express *c-myc* (reviewed in 1). *c-myc* expression is strictly dependent on mitogenic signals, is suppressed by growth-inhibitory signals and inducers of differentiation, and is important for proliferation and apoptosis in response to the appropriate stimuli (reviewed in 1, 2). The c-Myc protein (or Myc) conveys itself strong mitogenic and apoptotic stimuli.

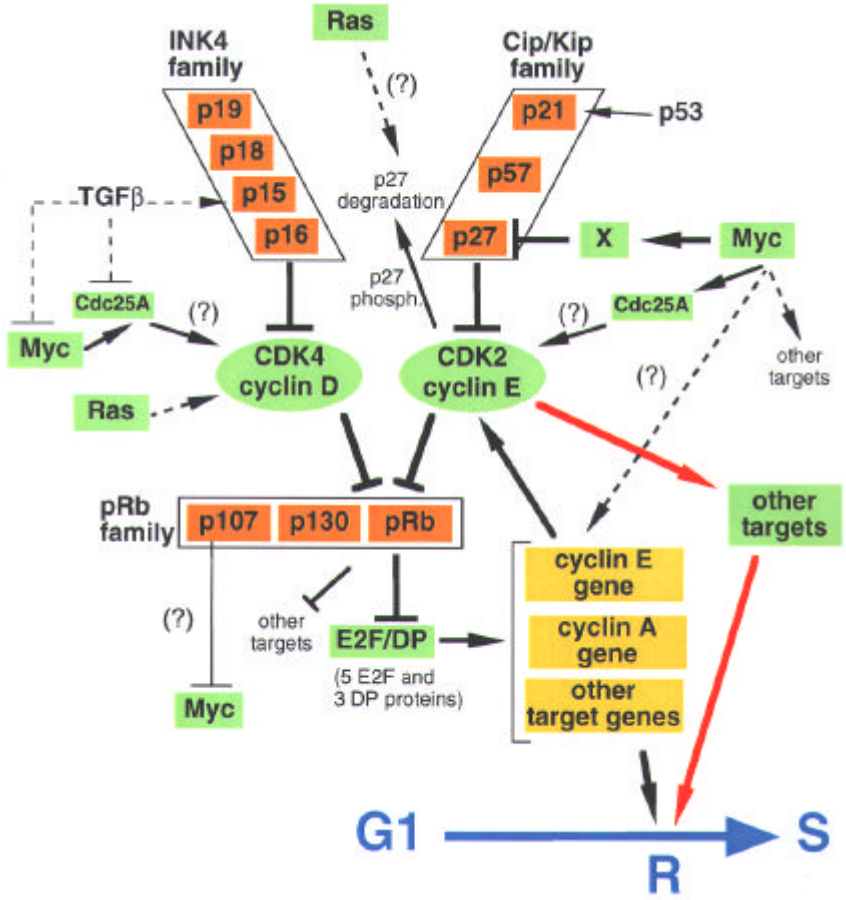

Figure 1. A partial and simplified representation of the molecular pathways regulating the G1-S transition in mammalian cells (see text for details). For the sake of simplicity, we have omitted the signalling pathways inducing the CKIs (INK4 and Cip/Kip families), with the exception of p53 and TGF-beta as examples. This scheme does not illustrate the specificity of interactions between individual pRb- and E2F-family proteins. Note that Cyclin A, which is expressed under the control of E2F-related factors and also associates with CDK2 and p27, has not been included in this scheme. Green boxes: positive regulators of cell cycle progression. Orange boxes: negative regulators. Yellow boxes: E2F-target genes. The question marks indicate that uncertainties persist on the role of Cdc25A in Myc-induced activation of CDKs, on the biological significance of the p107-Myc interaction, on the mechanisms underlying induction of cyclin E expression by Myc and on the role of Ras in p27 degradation.

Constitutive expression of Myc reduces growth-factor requirement, prevents growth arrest by a variety of growth-inhibitory signals, and can block cellular differentiation (e.g. 5-7; reviewed in 1). Conversely, activation of a conditional Myc-estrogen receptor chimaera (MycER; 8) in quiescent cells induces entry into the cell cycle in the absence of mitogens (9). However, concomitantly with their mitogenic action, either Myc or MycER induce apoptosis if survival factors are missing from the extracellular environment (10-12; reviewed in 1, 2).

Myc is a transcription factor of the basic-helix-loop-helix-leucine zipper (bHLH-Zip) family. Myc must dimerize with another bHLH-Zip protein, Max, to bind the specific DNA sequence CACGTG (the E-box) and activate transcription from adjacent promoters (e.g. 13, 14; reviewed in 2, 15). Transcription-competent Myc/Max dimers are the active form of Myc in inducing cell-cycle progression, apoptosis, and malignant transformation (2, 15-17). Max also forms heterodimers with the bHLH-Zip proteins Mad1, Mxi-1 (or Mad2), Mad3, Mad4 and Mnt (or Rox)(18-22). These alternative dimers bind the E-box and actively repress transcription, and can therefore antagonize both the transcriptional and transforming activities of Myc. In addition, *myc* and *mad* genes are generally regulated in opposite modes in growth control and development (e.g. 20, 23, 24; reviewed in 2). In summary, Myc, Max and Mad proteins form a network that regulates gene expression, proliferation, apoptosis and differentiation. One major question lies now in the identification of the target genes of this network. Only few of these genes have been identified, including alpha-prothymosine (9, 25), ornithine decarboxylase (ODC; 26), Cdc25A (27) (section 3.3.) and several others (reviewed in 2, 28). ODC and Cdc25A share some transforming and

apoptotic activities with Myc and may mediate Myc-induced apoptosis (27, 29-33).

In addition to its function as a transactivator, Myc has also been shown to repress transcription of several genes (reviewed in 2, 34). However, demonstration that this is a direct action of Myc *in vivo*, or relevant to its biological activity, is missing to date. Several possible mechanisms for transcriptional repression by Myc have recently been proposed (34-36), which will hopefully allow to address these questions.

The scope of the present review is to discuss recent studies addressing the mechanisms by which Myc influences cell cycle control. We will not exhaustively review the diverse cellular systems in which Myc was shown to prevent cell cycle arrest and differentiation, except for those which provide mechanistic cues. Emphasis will be given to the functional interactions between Myc and cell cycle-regulatory proteins such as cyclins, CDKs and CKIs. In doing so, we will venture into aspects of cell cycle control seemingly unrelated to Myc, but which we believe are relevant to its action. We will not discuss in detail the function of Myc- and Mad-family proteins in development, apoptosis and transcription, the control of Myc expression and, with few exceptions, Myc-interacting proteins or Myc-target genes. Recent reviews have covered these topics (2, 28, 34, 37) , to which the reader is referred. The field under survey here is still in its infancy, and subject to ample debate: the existing data support several preliminary, alternative, but also non-exclusive models, which must be considered at present as working hypotheses. Prior to entering this debate, however, a rapid survey of the relevant cell cycle-regulatory mechanisms is required.

2.2. G1-S control in mammalian cells: an outline

In vertebrate cells, the commitment to complete a round of mitotic division takes place during the initial phase of the cell cycle (G1), at a stage called the restriction (R-) point, preceding the onset of DNA synthesis (S phase; figure 1) (38, 39). The best characterized molecular event required for passage through the R-point is inactivation by phosphorylation of the Retinoblastoma protein (pRb). The cyclin-dependent kinases (CDKs) CDK4 or CDK6 (associated with D-type cyclins) and CDK2 (associated with cyclins E or A) phosphorylate pRb (40-43) and the related proteins p107 and p130 (44-48) (figure 1). pRb-family proteins (also called "pocket proteins") are negative growth-regulators; in their active form, they associate with transcription factors of the E2F family and repress their target genes, which include regulators of S-phase entry (e.g. B-*myb*, cyclins E and A) and genes required for DNA replication (e.g. DHFR, DNA pol alpha) (41, 49-51). Five E2F proteins (E2F-1 to 5) and three DP proteins (DP-1 to 3) have been cloned. Heterodimerization between E2F and DP is required for efficient DNA binding, transcriptional activity, and binding to pocket proteins (52-54). E2F-1, -2 and -3 bind predominantly pRB. E2F-4 binds all known pocket proteins and E2F-5 binds predominantly p130 (47, 48, 51, 55). CDK-mediated phosphorylation inactivates pocket proteins, allowing release of E2F and progression into S-phase.

The activity of CDKs is regulated by various mechanisms including association with cyclins, phosphorylation/dephosphorylation and association with two families of inhibitory proteins collectively called CKIs (reviewed in 56, 57) (figure 1). The first family includes $p21^{Cip1}$, $p27^{Kip1}$ and $p57^{Kip2}$, which bind all G1-cyclin/CDK complexes, but may primarily act as CDK2 inhibitors (58). The second family includes $p16^{INK4a}$, p15INK4b, $p18I^{NK4c}$ and $p19^{INK4d}$, which inhibit CDK4 and CDK6. CKIs play key roles in the response of cells to growth-inhibitory signals, such as induction of differentiation, p53 activation (e.g. after DNA damage), TGF-beta-induced growth arrest, contact-inhibition, senescence, and others (57, 59). As expected from these properties, ectopic expression of CKIs causes cell cycle arrest in G1.

Several studies suggested that one major role of cyclin D/CDK4 complexes is to inactivate pRb. For example, pRb-negative cells become insensitive to expression of p16 or microinjection of antibodies neutralizing cyclin D/CDK4 (60-64). Also, ectopic expression of E2F prevents G1 block by p16 (65-67). In contrast, several observations imply that cyclin E/CDK2 must have an additional role, distinct from pRb phosphorylation and E2F activation (68-73). This role, however, remains to be defined. Cyclin E is not only an upstream regulator of E2F (via pRb phosphorylation), but also its target, since the cyclin E gene is itself under the control of E2F and pRb (74-80). Thus, E2F and cyclin E are part of a positive feedback loop triggering passage through the R-point (figure 1).

The G1-regulatory pathway defined by p16, CDK4, D-type cyclins, pRb and E2F is critical in preventing oncogenic transformation and appears to be deregulated in most, if not all human malignancies. Individual genes of this pathway are altered in many different tumor types, resulting either in oncogenic activation (cyclins D1, D2, and CDK4) or loss-of-function (Rb and p16) (e.g. 81, 82 ; reviewed in 83, 84). Mice lacking the p16 gene develop multiple neoplasias (85). However, this phenotype might be attributable to the loss of $p19^{ARF1}$, an unrelated cell cycle-inhibitory protein encoded by the same locus (86, 87). Further studies will be required to elucidate the specific role of p16 in the mouse knock-out phenotype.

3. MYC POSITIVELY REGULATES CDK FUNCTION THROUGH SEVERAL PATHWAYS

Deregulated Myc expression prevents cell cycle withdrawal in response to antiproliferative stimuli such as TGF-beta, p53 activation, or inducers of differentiation (see introduction). In order to explain the action of Myc in molecular terms, it will be important to understand at what level(s) Myc interferes with those signals. The data reviewed in this section suggest that Myc up-regulates, and prevents inhibition of cyclin E/CDK2 activity, via at least three different pathways (figure 1): (i) inactivation of the CKI $p27^{Kip1}$ through the induction of an as yet unidentified p27-sequestering protein(s), which probably also targets p21Cip1 and $p57^{Kip2}$; (ii) induction of Cdc25A transcription; (iii) deregulation of cyclin E transcription by an as yet unkonwn and most likely indirect mechanism. In different cell types, physiological conditions or developmental stages, either one or a combination of these mechanisms may be rate-limiting

for Myc action, and may also influence the activity of cyclin D/CDK4 and cyclin A/CDK2 complexes.

3.1. Myc antagonizes the function of $p27^{Kip1}$

3.1.1 Constitutive expression of Myc prevents p27-induced growth arrest

Because CKIs are important mediators of antiproliferative signals (see introduction), we postulated that Myc may interfere with their growth-inhibitory function. To test this hypothesis, we developed a retroviral infection protocol allowing sequential expression of Myc and any CKI in cultured cells, and biochemical analysis of these cells shortly after infection. In the first part of our work (88), we showed that constitutive Myc expression abrogated the growth-suppressive function of p27. This was mediated by the non-covalent sequestration of p27 within cells into a form unable to bind and inhibit cyclin E/CDK2. However, Myc did not directly bind p27, implying that a distinct cellular protein(s) was mediating its action. Cyclin D/CDK4 (or CDK6) were plausible candidates, since these complexes had been shown to compete with cyclin E/CDK2 for p27 binding (57, 89-92). However, Myc did not increase association of p27 with cyclins D1-3 or CDK4. In addition, when expressed from retroviruses (alone or in several combinations) none of these cyclins prevented p27-induced arrest. Similar arguments ruled out cyclin A as mediatior of Myc action. Finally, inactivation of p27 required transcriptionally-active Myc/Max dimers. We thus postulated that Myc induces expression of a distinct p27-sequestering protein(s) (88) ("X" in figure 1). If the proposed model was correct, overexpression of p27 should saturate the sequestering protein(s) *in vivo* and arrest Myc-expressing cells. This prediction was fulfilled. First, the p27 retrovirus used in the above studies expressed physiological levels of p27, comparable to those of endogenous p27 induced by contact inhibition (88). When p27 was overexpressed as little as three to four-fold, it became dominant and induced G1 arrest in Myc cells as well as in controls (manuscript in preparation). Similarly, microinjection of plasmids encoding p27 or p21 blocked MycER-induced cell cycle entry (93). Thus, Myc only promotes growth in the presence of physiological p27 levels. We recently developed a direct *in vitro* assay for the p27-sequestering activity: when purified recombinant p27 was incubated with lysates from Myc-expressing cells, it became unable to subsequently inhibit a recombinant cyclin E/CDK2 complex. However, recombinant p27 was reactivated by boiling following incubation with the lysate, showing that a p27-sequestering activity was present in the lysate. This activity was dependent upon ectopic Myc expression in the cells used to prepare the lysate, was only detected upon incubation of p27 with the lysate prior to addition of the target cyclin E/CDK2 complex, and was distinct from previously known cyclins (JV and BA, unpublished data). This assay should allow further characterization of the Myc-induced p27 antagonist.

The ability of Myc to antagonize p27 function may extend to the related CKIs p21 and p57. First, Myc overcomes G1 arrest in response to p53 activation in fibroblasts (6) or differentiation stimuli in myeloid cells (7) without suppressing the associated induction of p21. Cyclin E/CDK2 complexes remained active in fibroblasts expressing Myc and active p53, and functional p21 could be recovered by boiling cell lysates, indicating that it might have become sequestered like p27 (6). One problem in interpreting these results is that the G1-arrest induced by p53 and differentiation inducers may require the products of other genes which are induced concomitantly with p21, such as gadd45 or the related protein MyD118, gadd34/MyD116, and others (94-96). In transient transfections, co-expression of GADD45 and p21, GADD45 and MyD118, or MyD118 and p21 suppressed colony outgrowth more efficiently than either protein alone (95-97). Myc was shown to reduce expression of gadd45 (98). Thus, it remains unclear whether Myc overcomes p53- or differentiation-induced arrest by antagonizing p21, other growth-inhibitory proteins, or both. We recently performed double-infection experiments with Myc and p21 or p57, and observed that Myc suppresses growth arrest by both CKIs (C. Gaillard, JV and BA, unpublished data). Whether this occurs by a mechanism analogous to the one described for p27 is currently under investigation.

3.1.2. Activation of Myc induces cell cycle entry by suppressing p27 function

The initial observation that activation of MycER in quiescent fibroblasts induced cell cycle entry (9) offered a tool to analyse the underlying molecular events. Work in Martin Eilers' group showed that MycER rapidly activated the catalytic function of preassembled cyclin E/CDK2 complexes, leading to hyperphosphorylation of pRb (99). Consistent with these findings, MycER induced E2F activity and deregulated expression of E2F-target genes, such as cyclins A and E (100). Activation of cyclin E/CDK2 was a prerequisite for the induction cyclin A (93), which is thus an indirect effect of Myc. Induction of cyclin D/CDK4 activity was delayed in this system in comparison to activation of cyclin E/CDK2 and hyperphosphorylation of pRb (99). Thus, MycER-induced cell cycle entry was largely driven by cyclin E/CDK2 activity. The functional target of MycER in activation of cyclin E/CDK2 was p27, the major inhibitor present in quiescent Rat1 cells. Indeed, MycER induced dissociation of p27 from cyclin E/CDK2 (101, 102) (JV and BA, unpublished data), and this effect was mediated by non-covalent sequestration of p27 into a form unable to bind cyclin E/CDK2 (101) (JV and BA, unpublished data), analogous to the conclusion from our studies on wild-type Myc (88) (section 3.1.1.).

Two additional observations made during the above studies generated some confusion and call for further investigation. The first concerns the relationship between Myc-induced p27 sequestration and p27 degradation, since both events follow activation of MycER in quiescent cells (99). Degradation of p27 was proposed to occur through the ubiquitin/proteasome pathway (103) and was triggered by phosphorylation of p27 on Thr 187 by CDK2 (104, 105). Several observations suggested that p27 degradation might be mechanistically unlinked from Myc-induced sequestration, and might be an indirect consequence of MycER-induced cell cycle entry. First, p27 degradation was a relatively late event following MycER activation. Second, Myc overrode p27-induced arrest without decreasing p27 levels or half-life in cells. Third, growth-rescue by Myc did not require phosphorylation of p27 on Thr 187, since it was not affected by mutation of this residue (JV and BA, unpublished data). A recent study, nonetheless, suggested that the two p27-

regulatory mechanisms might be linked (102) : phosphorylation of p27 might facilitate its release from cyclin E/CDK2, and free p27 might subsequently become a target for Myc-induced sequestration and degradation. In addition, the "sequestered" state was envisaged to be an intermediate in the degradation pathway. But why, then, would p27 phosphorylation be obligatory for degradation (104, 105), but not for the effect of Myc? It appears likely that phosphorylated Thr 187 constitutes a specific recognition motif for some component of the degradation machinery. The conditions under which p27 is phosphorylated on Thr 187 *in vivo* also remain unclear: this might occur within ternary complexes (which might be either a constitutive, or regulated event) or, alternatively, might require free cyclin E/CDK2 complexes acting in *trans*. It would not be productive at present to attempt proposing a new, unified model. Instead, the stage is set for further experimental work, aiming in particular at (i) understanding under which physiological conditions Thr 187 becomes phosphorylated *in vivo* and (ii) identifying the cellular proteins interacting with p27 in response to Myc activation or Thr 187 phosphorylation.

3.2. Myc up-regulates cyclin E expression, but what is the mechanism?

The second confusing point which arose from the MycER studies (see 3.1.2) concerns the regulation of cyclin E by Myc. Indeed, activation of MycER increased the expression of cyclin E mRNA and protein (100, 101, 106). This effect was rapid, occurred at the transcriptional level, and appeared to require no intervening protein synthesis (101). While these observations would suggest that cyclin E may be a direct transcriptional target of Myc, there is as yet no formal evidence supporting this conclusion (either from the sequence, or from direct studies of the cyclin E promoter). Since the cyclin E gene is an E2F target (figure 1), indirect mechanisms may be envisaged. Further studies must resolve this question.

Regardless of the mechanisms underlying the induction of cyclin E transcription by Myc, it can already be concluded that this effect cannot solely account for the effects of MycER or Myc on cell cycle progression and p27 sequestration or degradation. First, induction of an exogenous cyclin E gene in quiescent fibroblasts did not allow cell cycle entry: cyclin E associated with CDK2 but the complex was inhibited by p27, and MycER activation was still required for kinase activity (101, 102). Second, retroviral expression of human cyclin E together with p27 in Rat1 cells led to the formation of catalytically inactive ternary complexes with CDK2. Co-expression of Myc was still required to maintain cyclin E/CDK2 active in the presence of p27, and to prevent G1 arrest (88). Third, retrovirally expressed p27 remained stable in the presence of cyclin E (88, 105), as opposed to what observed with transient transfection of the two genes (104). Thus, at physiological expression levels (as achieved with retroviral vectors), cyclin E was insufficient to overcome the inhibition of CDK2 and trigger p27 degradation. This suggests that additional signals are required to allow phosphorylation of p27 by CDK2 and its subsequent degradation.

3.3. Myc target genes and cell cycle control: cdc25A may be one, others are still missing

Phosphatases of the Cdc25 family (in mammalian cells: Cdc25A, B and C) activate CDKs by dephosphorylating conserved residues (Thr14 and Tyr15 in CDC2 and CDK2; reviewed in 56). Myc was shown to induce transcription of the *cdc25*A gene, and expression of antisense (AS) *cdc25A* mRNA reduced Myc-induced apoptosis (27). This result was in apparent contradiction with the lack of a requirement of CDK2, 4, and 6 activity for Myc-induced apoptosis (93). Two hypotheses may explain this paradox: first, the target of Cdc25A in inducing apoptosis may not be CDK2, CDK4 or CDK6, but another, yet undetermined kinase. CDK3 is a possible candidate. Second, dephosphorylation of CDK2 or CDK4/6 may constitute a specific apoptotic signal. One problem with the antisense studies, however, is that they entailed the selection of cells stably expressing AS *cdc25A* mRNA (27). Since Cdc25A is normally required for cell cycle progression (107), this selection step may have introduced unknown compensatory changes. Thus, clarification of this issue will require new experimental systems, such as inducible expression of AS *cdc25*A mRNA.

What might be the role of Cdc25A in cell cycle regulation by Myc? The effects of Myc on p27 function (see 3.1.) clearly involve targets distinct from *cdc25A*. First, in our studies, the steady-state levels of Cdc25A mRNA and protein were not increased in Myc-expressing cells. Infection of Rat1 cells with a retrovirus encoding Cdc25A, resulting in expression of the protein 5-10 fold above endogenous levels, did not rescue p27-induced arrest (88) (S. Hennecke, JV and BA, unpublished). Second, Myc activity did not change the susceptibility of cyclin E/CDK2 complexes to inhibition by p27 (88, 102). Thus, the effects of Myc involved neither modification of CDK2 by Cdc25A, nor competition between Cdc25A and p27 for association with CDK2, as proposed for p21 (108). Third, cyclin E/CDK2 complexes, following their derepression by MycER *in vivo*, could be further activated by Cdc25A *in vitro* (99, 102). Thus, Cdc25A was still limiting for full CDK2 activity following MycER activation. Fourth, p27-inhibited complexes could not be activated by Cdc25A (102), showing that repression of cyclin E/CDK2 by p27 is dominant over activation by Cdc25A. Altogether, these observations imply that induction of Cdc25A and derepression of CDK2 in the presence of p27 are mechanistically distinct and separable effects of Myc. Thus, we still need to identify the physiological conditions under which Cdc25A might be a rate-limiting effector of Myc action in cell cycle control. In certain epithelial cells, the functional targets of Cdc25A might be CDK4 and CDK6, rather than CDK2 (109) (figure 1), and induction of Cdc25A by Myc may possibly play a role in overcoming TGF-beta-induced arrest (section 4.2).

Recently, the Cdc25A gene was also shown to be transcriptionally activated by E2F (K. Helin, personal communication). This will complicate the analysis of Cdc25A as a Myc target, since Myc can indirectly induce expression of E2F target genes. It is also conceivable that Myc and E2F may synergize in inducing Cdc25A expression, but this remains to be experimentally addressed.

3.4. The effects of decreasing or increasing Myc activity in growing cells

Exponentially growing cells express Myc at low levels. If Myc is limiting for the activity of cyclin E/CDK2 complexes, overexpression of dominant-negative (DN-) alleles of Myc would be expected to suppress this activity. Conversely, increasing Myc levels should lead to premature and increased activation of cyclin E/CDK2. As discussed below, both predictions were recently confirmed experimentally. First, transient transfection of a DN-Myc allele called MadMyc (a chimaeric construct combining the transcriptional repression domain of Mad1 with the bHLH-Zip domain of Myc) led to a reduction in cyclin E/CDK2 activity, without modifing the cellular levels of cyclin E, CDK2, or p27 (110). This effect was not due to reduced Cdc25A expression. It is thus likely that DN-Myc was leading to association of p27 with cyclin E/CDK2. The experimental system used did not y*et al*low direct testing of this prediction.
A second report described the effects of MycER activation in growing cells, using size-fractionation of cell populations by elutriation (106). In control cells (i.e. cells expressing no, or inactive MycER), activation of cyclin E/CDK2 and cyclin A/CDK2, disappearance of p27 and hyperphosphorylation of pRb all occurred in late G1, after cells reached a critical cell size. In the presence of active MycER, these events occurred in small early-G1 cells, just after exit from mitosis. Thus, Myc activity in proliferating cells was rate-limiting for activation of cyclin E/CDK2 (and, consequently, cyclin A/CDK2; 93). Strikingly, however, MycER activation did not significantly advance the timing of S-phase entry in those cells (106). The authors proposed that additional, Myc-independent mechanisms operate to ensure that DNA replication is not initiated before cells have reached the appropriate size. Consistent with this conclusion, proliferating fibroblasts expressing Myc ectopically did not show obvious changes in cell cycle distribution compared with control cells (e.g. 72, 88). It should be noted, however, that Myc overexpression can also shorten G1 in some cells (111). This may reflect the fact that Myc is rate-limiting not only for CDK2 activation, but also for cell growth *per se*, as discussed further below (section 6.).

In summary, the data discussed so far show that cyclin E/CDK2 is a functional target of Myc. Myc may operate at several levels to up-regulate the kinase activity of this complex, including the inactivation of CKIs (p27 and probably p21 and p57), the induction of the CDK-activating phosphatase Cdc25A, and the up-regulation of cyclin E expression (most likely through an indirect mechanism). The two former mechanisms would also be expected to contribute to up-regulation of cyclin D/CDK4 activity by Myc, although direct evidence for this is still missing.

4. MYC AND THE p16-pRb PATHWAY

4.1. Cyclin E, the spy who came in from the cold

Using the same system as that described for p27 (88) (section 3.1.1), we showed that Myc also suppresses growth arrest by CKIs of the INK4 family, and in particular by $p16^{INK4a}$ (72). Studying the mechanisms underlying this effect led to two unexpected observations. First, cyclin E (which is a target of p27 but not p16; figure 1) also prevented p16-induced arrest. Second, p16 was still functional in Myc- or cyclin E-expressing cells, since it associated with its target CDKs and induced dephosphorylation and activation of pRb, p107 and p130. In summary, expression of either Myc or cyclin E dissociated activation of p16 and pRb from growth arrest.

We propose that cyclin E is the effector of Myc in bypassing p16 and pRb function in G1-S progression. As discussed above, Myc positively influences cyclin E/CDK2 function via any of three mechanisms: inactivation of p27 (and possibly p21 or p57), and up-regulation of Cdc25A and cyclin E expression (note, however, that overexpression of Cdc25A could not substitute for Myc or cyclin E in bypassing growth arrest by p16 (72), suggesting that Cdc25A was not the rate-limiting factor for cyclin E/CDK2 activity in p16-arrested Rat1 cells). In summary, the net effect of Myc action is to increase cyclin E/CDK2 activity which, in turn, supports cell proliferation in the presence of p16 and activated pRb. Thus, we must now attempt to explain the action of cyclin E.

A pRb-independent role for cyclin E (in addition to its function as a pRb kinase; figure 1) had been suggested by previous studies (68-70) Our work on p16, summarized above (72), and two independent and parallel studies (71, 73) further showed that cyclin E can promote S-phase entry in the presence of active pRb. Using transient transfection and/or microinjection, these other authors showed that cyclin E bypassed G1 arrest induced by constitutively active pRb mutants (lacking CDK-phosphorylation sites). In one of these studies (71), cyclin E also bypassed G1 arrest by a dominant-negative DP-1 mutant which blocked E2F function. While this result suggests that cyclin E might be the sole rate-limiting target of E2F, it is in apparent direct contrast with studies showing that adenovirus-mediated expression of E2F-1 allowed cell cycle progression in the absence of CDK2 and CDK4 activity (65). In summary, high-levels of cyclin E or E2F activity, each in the absence of the other, suffice to drive a single cell cycle (or at least S-phase). However, this is not *a priori* true with physiological levels of cyclin E or E2F. For example, moderate expression of E2F-1 did not suffice to bypass p27-induced arrest (and thus cyclin E/CDK2 inhibition), whereas it readily overcame p16-induced arrest (66, 72). How can we rationalize these findings? The most likely explanation is that cyclin E and E2F might each have a non-redundant, essential role in G1-S progression in mammalian cells, as was concluded from experiments in *Drosophila* (112-114). In transient transfection experiments (e.g. 71), only one pathway (i.e. cyclin E) might have been rate-limiting for S-phase entry, the second (i.e. E2F) having been already activated to sufficient levels prior to the beginning of the experiment. This explanation would also apply to the converse experiment (65). In summary, under physiological conditions, cyclin E and E2F activity cross-regulate each-other but may also be independently required for S-phase entry.

How is this discussion relevant to the present review? Cyclin E is a key functional target of Myc, and both proteins can bypass activation of the p16-pRb pathway. One additional effect of Myc, which remains to be investigated, may be to directly induce expression of E2F-family members

(115, 116), providing an additional route not only to bypass growth arrest by p16 and pRb, but also to induce cyclin E. We now need to identify the molecular pathway(s) and substrates through which cyclin E/CDK2 bypasses growth arrest by p16 and pRb (figure 1, red lines).

4.2. Cellular context may determine the ability of Myc to promote cell cycle progression

In apparent contrast with our studies on p16 (72), microinjection of p16-encoding plasmids blocked MycER induced cell cycle-entry (but not apoptosis) (93). How do we reconcile these observations? Other studies showed that cyclin D/CDK4 complexes can serve as a reservoir for p27, and that INK4-family proteins can displace p27 from these complexes, allowing p27 to inhibit cyclin E/CDK2 (90, 117). Thus, INK4 proteins can serve a dual role in inhibiting directly CDK4 and CDK6 and, indirectly, CDK2. In our experiments (72), p16 or p15 were introduced by infection in proliferating cells, which expressed low levels of p27 and no detectable p21. Thus, in this system, the "p27-displacement" mechanism did not play a major role in growth arrest induced by p16 or p15 (indeed, endogenous cyclin E activity, although it was not sufficient to sustain growth, was not fully repressed in p16-arrested cells). In the microinjection experiments (93), p16 was introduced in contact-inhibited, serum-starved cells, in which endogenous p27 was maximally expressed. Under these conditions, p27 is distributed between cyclin D/CDK4 and cyclin E/CDK2 complexes, with a large fraction on the former. Thus, overexpression of p16 in those cells is expected to displace p27 from cyclin D/CDK4, significantly enhancing the pool of free p27. This may generate a situation in which elevated free p27 levels negate the ability of Myc to overcome inhibition of cyclin E/CDK2 (section 3.1.1.). In summary, we propose that endogenous p27 cooperated with microinjected p16 in preventing MycER-induced cell cycle entry. This hypothesis emphasizes the notion that the decision to arrest or divide rests on a very fine balance, determined by the combination and strength of various growth-inhibitory and stimulatory pathways. Whether or not Myc (or MycER) is dominant over growth arrest will be exquisitely dependent on cellular context.

It appears now important to go back to analyse the mechanisms underlying Myc action in the presence of physiological growth-inhibitory stimuli. As an example, ectopic Myc expression can prevent growth arrest in response to TGF-beta treatment (5). TGF-beta-induced arrest in epithelial cells relies on two independent pathways. The first is the "p27-displacement" mechanism outlined above, in this case via the induction of $p^{15INK4b}$ (89, 90, 117, 118) (figure 1). The second pathway is the down-regulation of Cdc25A expression (most likely at the transcriptional level), which leads to inactivation of CDK4 and CDK6 (109) (figure 1). TGF-beta also down-regulates Myc expression in those cells (109, 119) (note, however, that TGF-beta-mediated inhibition of transcription from the Cdc25A promoter did not require the Myc-response elements (109), indicating that a Myc-independent mechanism was involved). We propose that deregulated Myc expression may antagonize this physiological growth-inhibitory stimulus via both induction of Cdc25A and sequestration of p27 (figure 1). This, in turn, may further contribute to bypassing the growth-inhibitory effect of p15. In addition, it remains to be asked whether Myc has any effect on the induction of p15 by TGF-beta.

4.3. Relationship between Myc and cyclin D1 in mitogenic signalling

The expression of D-type cyclins, like that of Myc, is regulated by mitogenic stimuli (43). For example, the cyclin D1 gene was initially cloned by virtue of its induction by CSF-1 (120). Myc is also induced upon activation of the CSF-1 receptor (CSF-1R). Since *c-myc* is an immediate-early response gene, and cyclin D1 is induced in a delayed-early fashion by mitogens, it was tempting to speculate that cyclin D1 might be a direct transcriptional target of Myc. However, an initial report arguing in this direction (121) was later shown to be flawed by the use of MycER under inappropriate experimental conditions (122). Instead, Myc down-regulates cyclin D1 transcription in certain cell types (123, 124). At the same time, Myc might increase translation of the cyclin D1 mRNA through up-regulation of the translation factor eIF-4E (125-127). Most likely, none of these observations reflects the true physiological relationship between Myc and cyclin D1 expression, which might be more complex and depend on the specific mitogenic stimuli encountered by cells. A mutant of CSF-1R failing to induce Myc, CSF-1R (Y809F) (128), also failed to induce cyclin D1 (129). CSF-1R (Y809F) was defective in mitogenic signalling, but ectopic expression of either Myc or cyclin D1 restored its mitogenic action (128, 129). Furthermore, Myc expression restored cyclin D1 induction by CSF-1R (Y809F), and vice-versa (128, 129). Thus, Myc and cyclin D1 are both required for mitogenic signalling and do not lie on a linear pathway, but are interdependent for their induction in response to CSF-1R activation. It should be noted that cyclin E expression rescued neither cyclin D1 induction, nor mitogenic signalling by CSF-1R (Y809F) (129), indicating that Myc did not act simply by increasing cyclin E levels. Although activation of cyclin E/CDK2 complexes might still have been involved (through either derepression from p27 or induction of Cdc25A), it is likely that a different effect of Myc was responsible for the co-induction of cyclin D1.

5. MYC AS A DOWNSTREAM TARGET OF POCKET PROTEINS AND E2F

We have discussed so far the function of Myc as an upstream regulator of CDKs and pRb-family proteins. However, Myc function may itself be regulated by those proteins in at least two ways, providing possible feedback mechanisms.

First, transcription of the c-myc gene may be regulated in part by E2F (e.g. 130-133). *c-myc* transcription is induced by a variety of receptor- and cytoplasmic tyrosine kinases, and different - somewhat contrasting - signalling pathways were proposed to be involved (e.g. 134-139). Growth-inhibitory stimuli or growth-factor starvation suppress *c-myc* expression, and this may occur via pocket proteins (e.g. 119, 140). However, unlike other E2F-target genes, *c-myc* was not induced by overexpression of E2F-1 (79), loss of pRb, or combined deletion of p107 and p130 (78). Under which physiological conditions E2F and pocket proteins are critical for c-myc transcription - if at all - remains

unclear at present, and will not be discussed further. Second, p107 has been shown to bind the transactivation domain of the Myc protein and to repress its activity (141, 142). Lymphoma-derived mutations in the Myc transactivation domain may allow escape from this repression effect, but this remains controversial (143, 144). Based on transfection studies, it was recently proposed that p16 could suppress the transactivation and transfoming function of Myc, and that this effect might be mediated by p107 (145). Thus, Myc may be a downstream target of p107 in the same way as E2F: cyclin D/CDK4 would be required to release p107 and allow Myc activity, whereas p16 would antagonize this effect (figure 1). How do we reconcile these findings with the ability of Myc to bypass p16-induced arrest (72) (section 4.1.)? As a possible explanation, ectopic activation of a downstream effector can bypass a growth-inhibitory pathway, as exemplified by the ability of E2F-1 to suppress both pRb- (146, 147) and p16-induced G1 arrest, in conditions in which cellular pRb remains hypophosphorylated (66, 67, 72). However, Myc was not largely overexpressed in our studies, making titratrion of p107 an unlikely mechanism for the overcome of p16 action (discussed in 72). Further work will be required to elucidate whether and under what conditions the p107-Myc interaction is limiting for cell cycle progression.

6. LESSONS FROM A MYC KNOCK-OUT CELL LINE: INDEPENDENT EFFECTS ON CELL CYCLE AND CELL GROWTH

Several experiments have suggested that Myc function is essential for cell proliferation (1, 2). A recent paper has come to challenge this view, suggesting that loss of Myc function impairs, but does not abrogate cell proliferation (148). These authors eliminated both alleles of the c-myc gene (or myc) by homologous recombination in a diploid clone of Rat1 fibroblasts. Surprisingly, *myc*$^{-/-}$ cells divided and proliferated, but very slowly. N-*myc* or L-*myc* expression could in principle have been responsible for growth of *myc*$^{-/-}$ cells. However, the corresponding mRNAs were undetectable by RNAse protection. Furthermore, myc$^{-/-}$ cells were passaged continuously for extended periods of time without giving rise to any fast-growing phenotypic revertants, which could have arisen from amplification of N-or L-*myc*. The selective pressure in favour of such events is tremendous, and their absence further indicates that N-or L-*myc* were probably silent.

Analysis of cell cycle distribution revealed that *myc*$^{-/-}$ cells had elongated G1 and G2 phases, whereas the time required for completion of S-phase (and, presumably, mitosis) was normal (148). Why were both G1 and G2 affected in those cells? The authors provided a very attractive explanation, which is that loss of Myc had a generalized effect on cell growth. Indeed, *myc*$^{-/-}$ cells had decreased rates of total RNA and protein accumulation, as well as protein degradation, but the average cell size was normal. If cell-cycle checkpoints monitoring cell volume at the G1-S and G2-M transitions were intact in *myc* $^{-/-}$ cells, the expected phenotype would be exactly as seen: a temporal delay in G1 and G2 exit, ensuring that DNA synthesis and mitosis are only executed at the appropriate size. This function of Myc in cell growth does not contradict, but rather complements its specific role in cell cycle control. In the G1 phase, loss of Myc would delay both activation of cyclin E/CDK2 and cell growth, both effects contributing to delay S-phase entry. It is thus predictable that elevation of cyclin E/CDK2 activity alone may not shorten G1 in *myc* $^{-/-}$ cells, since cell volume would still increase slowly and cyclin E activity is insufficient to bypass the cell-size checkpoint, as shown by MycER-activation studies (section 3.4.) (106). In the G2 phase, *myc* $^{-/-}$ cells would grow slowly, extending the time required to reach the minimum appropriate size required to enter mitosis. A specific role for Myc in progression through G2 was also suggested (134), but remains to be established. Molecular analysis of the cell cycle defects in *myc* $^{-/-}$ cells guarantees several breakthroughs in the near future.

If Myc activity is rate-limiting for cell growth, a subset of Myc-regulated genes should be involved in biosynthetic pathways. We recently identified two new Myc-activated genes which encode BN51, a subunit of RNA-polymerase III (149, 150), and Nucleolin, a nucleolar protein most likely involved in ribosome biogenesis (e.g. 151) (P. Greasley and BA, unpublished data). BN51 is particularly interesting, since it was cloned on the basis of its ability to complement temperature-sensitive mutation causing G1 arrest in fibroblasts (152). It is important to remark here that active pRb suppresses transcription by RNA polymerases I and III (153-158). Thus, growth-regulatory signals may directly impinge on the activity of these polymerases, providing a major and general control mechanism on cellular biosynthetic pathways.

7. CELLULAR TRANSFORMATION AND ONCOGENE COOPERATION: THE EXAMPLE OF MYC AND RAS

Transformation of cells to an overt malignant phenotype requires multiple genetic lesions. One classical paradigm for *Oncogene* cooperation is the transformation of primary rodent fibroblasts by *myc* and *ras Oncogene*s and, in this review, we will confine ourselves to a survey of this phenomenon. Recent studies showed that *ras* also controls rate-limiting steps in cell cycle control (159), suggesting that the cooperation between *ras* and *myc* may be largely due to their complementary and synergistic action on CDK function.

7.1. Myc and Ras: interplay in cell cycle control

Depending on the cellular context, an activated Ras protein or its effector Raf (reviewed in 160) can induce cell cycle arrest (which will be discussed in the next section) or progression. Ras transduces mitogenic stimuli in response to tyrosine-kinase receptors, and its function is required in G1 for passage through the R-point (161, 162). Ras activity is required for the phosphorylation of pRb in response to mitogenic signalling, and functional inactivation of Ras induces G1 arrest in pRb-positive, but not in pRb-negative cells (115, 163, 164). The mitogenic signal mediated by Ras and Raf may act through the induction of cyclin D1 and/or the degradation of p27 (115, 163, 165-168) (figure 1). The mechanisms underlying the effects of Ras on p27 remain to be dissected. It is unclear, for instance, whether Ras acts in concert with CDK2-induced degradation of p27 (104, 105) or through a different pathway. In any case, the action of Ras

on p27 may add to that of Myc (88, 101, 102) (section 3.1.), both contributing to the derepression of cyclin E/CDK2. The activities of Myc and Ras/Raf may also complement at the level of Cdc25A, which might be activated directly by the Raf kinase (169). The expected result is a synergistic enhancement of cyclin E/CDK2 activity by Myc and Ras, as was shown using adenovirus-mediated expression of the two proteins (115). The notion that G1-CDK activities are key rate-limiting targets of mitogenic signals was recently substantiated by the finding that microinjection of preformed cyclin D1/CDK4 or cyclin E/CDK2 complexes (but not of cyclin A/CDK2) in quiescent cells induced S-phase entry in the absence of mitogens (170).

Another possible level of convergence between Ras and Myc is the translation factor eIF-4E. First, expression of eIF-4E is induced by Myc (125, 126). Second, the activity of the eIF-4E protein may be regulated by Ras-induced phosphorylation (reviewed in 171). Third, eIF-4E levels appear to be limiting for cellular transformation (172, 173). One of the critical roles of eIF-4E might be translation of the cyclin D1 mRNA (127), although more general effects on protein synthesis are also likely. eIF-4E was also shown to suppress Myc-induced apoptosis (174).

7.2. Interplay between tumor suppressor and oncogenic pathways

Expression of *myc* or activated *ras* alone cannot transform primary cells, but both together effectively do so (175, 176), and also cooperate in tumorigenesis *in vivo* in mice (e.g. 177-181). Myc, as well as the viral proteins Large T (LT) and E1A, are "immortalizing" oncoproteins which allow primary cells to bypass the senescence crisis and become established in culture. All these proteins cooperate with *ras* (reviewed in 182), consistent with the fact that cellular immortalization is a prerequisite (although it is not always sufficient) for full transformation by *ras* (183, 184). In primary cells and a minority of cell lines (e.g. REF52; 184), activated Ras or Raf do not promote proliferation but instead induce G1 arrest (185, 186) equivalent, at least in fibroblasts, to accelerated senescence (187). Ras/Raf activation induces the accumulation of active p53, thereby inducing p21 expression and CDK inhibition. LT or E1A overcome Ras/Raf-induced arrest (185-187). Whether Myc will overcome Ras/Raf-induced arrest in primary cells has not been reported (but see 188), although it is predictable given that these proteins cooperate in cellular transformation.

Functional inactivation of p53 or p16 can bypass senescence and allow cellular transformation by *ras* alone (85, 186, 187, 189-191). Homozygous deletion of either the p53 or p16 (INK4a) genes led to the development of multiple tumors in mice (187, 192), and loss of the INK4a gene cooperated with an activated *ras* transgene in the induction of melanoma *in vivo* (193). However, it is crucial to note here that the INK4a knock-out studied so far affected not only $p16^{INK4a}$, but also the alternative gene product $p19^{ARF1}$. A selective p19ARF1 knock-out reiterated the phenotypes previously attributed to p16-loss, including increased tumor incidence and direct cellular transformation by ras (87). Although the role of p16 as a tumor suppressor in humans is not under question (194), the specific contribution of p16-loss to cellular immortalization and transformation *in vitro*, and to tumorigenesis *in vivo*, remains to be assessed. Functional p53 was required for the cell cycle-inhibitory and senescence-inducing function of $p19^{ARF1}$ (87), suggesting that $p19^{ARF1}$ and p53 may lie on a common tumor suppressor pathway. This pathway may conceivably include the p53-mediated inducion of p21, since p21-null MEFs appeared to bypass the senescence crisis (195). However, p21-null animals did not display a tumor-prone phenotype (195), suggesting that other pathways, and in particular p53-induced apoptosis, are involved in suppressing tumorigenesis. In the context of the present review we can provisionally conclude that, in terms of immortlization and cooperation with ras, Myc activation phenotypically mimics loss of either p53 or $p16^{INK4a}$/$p19^{ARF1}$. Thus, the ability of Myc to overcome cell cycle arrest by p16-pRb or p53-p21 (as well as p27) (6, 72, 88) may explain in part, but not entirely, its oncogenic properties.

Several observations show that c-myc activation does not only mimic loss of the p16-pRb or p53-p21 growth-inhibitory pathways in tumorigenesis. For example, loss of p53 synergizes with Myc activation in lymphomagenesis (196-199). This may be due to independent effects on cell growth (198), or to decreased Myc-induced apoptosis in the absence of p53 (200, 201). The latter mechanism also underlies the cooperation between Myc and Bcl-2 in lymphomagenesis (202). In transgenic mice, cyclin D1 cooperated with Myc in lymphomagenesis (203, 204), suggesting that the two proteins also control distinct pathways. Moreover, apparently in contrast with our data on cell cycle progression (72), p16 overexpression suppressed cellular transformation by co-transfected myc and ras (205) or cyclin E and *ras* (206) (see below). This most likely reflects an effect of p16 on the action of Ras (e.g. by reversing Ras-induced inactivation of pRb) (115, 163, 164), or alternatively of Myc (145), or both. In summary, although activation of pRb is ineffective in inducing G1 arrest in the presence of Myc (or cyclin E) (72), it may still effectively suppress co-transformation by Myc and Ras. This emphasizes the important notion that cell cycle progression and cellular transformation, although connected, are two different biological and experimental outcomes.

Recently, several observations have linked the aberrant expression of cyclin E to tumorigenesis. For example, cyclin E cooperated with Ras in cotransfection assays (206) and had a mild oncogenic potential in transgenic mice (207). Moreover, aberrant cyclin E isoforms were detected in human tumor cells (e.g. 208, 209). It was suggested that in tumor cells that express high p16 levels, cyclin E/CDK2 could replace CDK4 function by phosphorylating pRb (210). However, the data discussed above (section 4.1.) establish that cyclin E can bypass p16-pRb function by an alternative pathway (71-73). The causal involvement of cyclin E in human tumors remains to be investigated. It is tempting, however, to speculate that deregulation of cyclin E may not fully substitute for Myc in tumorigenesis, either because cyclin E/CDK2 is still susceptible to inhibition by p27 (or p21, p57) unless Myc is activated (88, 101, 102), or because Myc has other functional targets, in particular cyclin D/CDK4, cyclin D/CDK6, as well as general biosynthetic pathways.

7.3. Do p21, p27 or p57 function as tumor suppressors?

Finally, the possible role of p21, p27 or p57 in suppressing tumorigenesis remains an open question. The genes encoding those CKIs cannot be classified, genetically, as tumor suppressor genes (195, 211-216) (although loss of p21 or p57 may contribute to the development of some tumors; 216-219). In spite of this, we would like to propose here that p27, p21 and/or p57 may play a central role in suppressing tumorigenesis. First, the lack of mutations in these proteins may be partly explained by functional redundancy. Second, the function and expression of p27 are altered by at least two oncogenic processes, activation of Myc (88, 101, 102) (section 3.1.) and Ras (115, 165, 168) (section 7.1., figure 1). Any one of these processes may simultaneously target p21 and/or p57. Third, low p27 levels are indicative of poor prognosis in mammary and colon carcinomas (209, 220, 221), which may result from increased proteolytic degradation of p27 in high-grade tumors (221). It will thus be fundamental to understand in detail how Myc-dependent and Ras-dependent mechanisms (i.e.: sequestration and proteolysis) may lead to loss of p27, and possibly of p57 and p21 function in cancer cells, liberating cyclin E/CDK2 activity from upstream cellular controls and promoting unrestrained growth.

8. PERSPECTIVES

The mechanisms by which Myc influences cell cycle control have become apparent over the last years. We are still lacking, however, a detailed description of these mechanisms. In particular, we will need to understand in the future precisely how Myc induces the inactivation of CKIs of the p21/p27 family, and the role of Cdc25A as a mediator of Myc action. In addition, new molecular targets of Myc will most likely emerge with the study of new cellular systems. All of the tested biological activities of Myc, including transformation, apoptosis, cell cycle progression and bypass of cell cycle arrest by p27 or p16, require the formation of transcriptionally active Myc/Max dimers (16, 17, 72, 88). Thus, the largest gap in our understanding of Myc function is, by far, our lack of knowledge about Myc-target genes. This gap must be filled-in from two sides: first, new and large-scale screens for Myc-regulated genes; second, identifying the cellular proteins which mediate the effects of Myc on the function of CKIs, CDKs, or other cell-cycle regulators.

Both Myc and Ras lie in a complex network of signaling pathways. Their activities may converge in regulating, through complementary and distinct mechanisms, the activities of functionally distinct proteins such as p27, Cdc25A, eIF-4E and others. The role of these proteins - and in particular of CKIs of the p27 family - in growth control and tumorigenesis is now an important focus of research.

Over the last years, it has become apparent that the deregulation of several major growth-regulatory pathways is instrumental in tumor progression. Among the most universal genetic defects in human tumors are alterations of the p16-pRb pathway and of p53. Other examples include the WNT-APC-beta catenin and the Sonic hedgehog-patched-smoothened signaling pathways (e.g. 222, 223). Through these discoveries, basic research identifies new molecular targets that translate into tools with potentially high prognostic, diagnostic and therapeutic value in oncology. However, therapeutic intervention at the molecular level must be evaluated case-by-case. For example, the restoration of p16 function may in principle be useful in tumors lacking p16, but will be totally ineffective in tumors which have lost pRb, or which have deregulated other downstream effectors of this pathway. This illustrates a strong case in favor of intervention at the level of downstream targets. Thus, the finding that Myc and cyclin E can promote cell proliferation by acting downstream of p16 and pRb (71-73) calls for additional research efforts in identifying the additional targets of these molecules. It is a safe prediction that the downstream effectors of Myc and, in particular, of cyclin E/CDK2 will be prime targets for intervention in a large spectrum of tumors.

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10. REFERENCES

1. Marcu K. B., S. A. Bossone & A. J. Patel: myc function and regulation. *Annu Rev Biochem* 61, 809-860 (1992)

2. Henriksson M. & B. Lüscher: Proteins of the Myc network: essential regulators of cell growth and differentiation. *Adv Cancer Res* 68, 109-182 (1996)

3. Garte S.: The c-myc *Oncogene* in tumor progression. *Crit Rev Oncogenesis* 4, 435-449 (1993)

4. Downs K. M., G. R. Martin & J. M. Bishop: Contrasting patterns of myc and N-myc expression during gastrulation of the mouse embryo. *Genes Dev* 3, 860-869 (1989)

5. Alexandrow M. G., M. Kawabata, M. Aakre & H. L. Moses: Overexpression of the c-Myc oncoprotein blocks the growth-inhibitory response but is required for the mitogenic effects of transforming growth factor ß1. *Proc Natl Acad Sci USA* 92, 3239-3243 (1995)

6. Hermeking H., J. O. Funk, M. Reichert, J. W. Ellwart & D. Eick: Abrogation of p53-induced cell cycle arrest by c-Myc: evidence for an inhibitor of p21WAF1/CIP1/SDI1. *Oncogene* 11, 1409-1415 (1995)

7. Steinman R. A., B. Hoffman, A. Iro, C. Guillouf, D. A. Liebermann & M. E. el-Houseini: Induction of p21 (WAF-1/CIP1) during differentiation. *Oncogene* 9, 3389-3396 (1994)

8. Eilers M., D. Picard, K. R. Yamamoto & J. M. Bishop: Chimaeras of myc oncoprotein and steroid receptors cause hormone-dependent transformation of cells. *Nature* 340, 66-68 (1989)

9. Eilers M., S. Schirm & J. M. Bishop: The MYC protein activates transcription of the alpha-prothymosin gene. *EMBO J* 10, 133-141 (1991)

10. Askew D. S., R. A. Ashmun, B. C. Simmons & J. L. Cleveland: Constitutive c-myc expression in an IL-3-dependent myeloid cell line suppresses cell cycle arrest and accelerates apoptosis. *Oncogene* 6, 1915-1922 (1991)

11. Evan G. I., A. H. Wyllie, C. S. Gilbert, T. D. Littlewood, H. Land, M. Brooks, C. M. Waters, L. Z. Penn & D. C. Hancock: Induction of apoptosis in Fibroblasts by c-Myc protein. *Cell* 69, 119-128 (1992)

12. Harrington E. A., M. R. Bennett, A. Fanidi & G. I. Evan: c-Myc-induced apoptosis in fibroblasts is inhibited by specific cytokines. *EMBO J* 13, 3286-3295 (1994)

13. Amati B., S. Dalton, M. W. Brooks, T. D. Littlewood, G. I. Evan & H. Land: Transcriptional activation by the human c-Myc oncoprotein in yeast requires interaction with Max. *Nature* 359, 423-426 (1992)

14. Kretzner L., E. M. Blackwood & R. N. Eisenman: Myc and Max proteins possess distinct transcriptional activities. *Nature* 359, 426-429 (1992)

15. Amati B. & H. Land: Myc - Max - Mad: a transcription factor network controlling cell cycle progression, differentiation and death. *Curr Opin Genet Dev* 4, 102-108 (1994)

16. Amati B., M. W. Brooks, N. Levy, T. D. Littlewood, G. I. Evan & H. Land: Oncogenic activity of the c-Myc protein requires dimerization with Max. *Cell* 72, 233-245 (1993)

17. Amati B., T. D. Littlewood, G. I. Evan & H. Land: The c-Myc protein induces cell cycle progression and apoptosis through dimerization with Max. *EMBO J* 12, 5083-5087 (1993)

18. Ayer D. E., L. Kretzner & R. N. Eisenman: Mad: a heterodimeric partner for Max that antagonizes Myc transcriptional activity. *Cell* 72, 211-22 (1993)

19. Zervos A. S., J. Gyuris & R. Brent: Mxi1, a protein that specifically interacts with Max to bind Myc-Max recognition sites. *Cell* 72, 223-232 (1993)

20. Hurlin P. J., C. Queva, P. J. Koskinen, E. Steingrimsson, D. E. Ayer, N. G. Copeland, N. A. Jenkins & R. N. Eisenman: Mad3 and Mad4: novel Max-interacting transcriptional repressors that suppress c-myc dependent transformation and are expressed during neural and epidermal differentiation. *EMBO J* 14, 5646-5659 (1995)

21. Hurlin P., C. Quéva & R. Eisenman: Mnt, a novel Max-interacting protein is coexpressed with Myc in proliferating cells and mediates repression at Myc binding sites. *Genes Dev* 11, 44-58 (1997)

22. Meroni G., A. Reymond, M. Alcalay, G. Borsani, A. Tanigami, R. Tonlorenzi, C. L. Nigro, S. Messali, M. Zollo, D. H. Ledbetter, R. Brent, A. Ballabio & R. Carrozzo: Rox, a novel bHLHZip protein expressed in quiescent cells that heterodimerizes with Max, binds a non-canonical E box and acts as a transcriptional repressor. *EMBO J* 16, 2892-2906 (1997)

23. Ayer D. E. & R. N. Eisenman: A switch from Myc:Max to Mad:Max heterocomplexes accompanies monocyte/macrophage differentiation. *Genes Dev* 7, 2110-2119 (1993)

24. Chin L., A. N. Schreiber, I. Pellicer, K. Chen, H. W. Lee, M. Dudast, C. C. Cordon & R. A. DePinho: Contrasting roles for Myc and Mad proteins in cellular growth and differentiation. *Proc Natl Acad Sci USA* 92, 8488-8492 (1995)

25. Gaubatz S., A. Meichle & M. Eilers: An E-box element localized in the first intron mediates regulation of the prothymosin alpha gene by c-myc. *Mol Cell Biol* 14, 3853-3862 (1994)

26. Bello-Fernandez C., G. Packham & J. L. Cleveland: The ornithine decarboxylase gene is a transcriptional target of c-Myc. *Proc Natl Acad Sci U S A* 90, 7804-7808 (1993)

27. Galaktionov K., X. Chen & D. Beach: Cdc25 cell-cycle phosphatase as a target of c-myc. *Nature* 382, 511-517 (1996)

28. Grandori C. & R. Eisenman: Myc target genes. *Trends Biochem Sci* 22, 177-181 (1997)

29. Galaktionov K., A. K. Lee, J. Eckstein, G. Draetta, J. Meckler, M. Loda & D. Beach: CDC25 phosphatases as potential human *Oncogenes*. *Science* 269, 1575-1577 (1995)

30. Auvinen M., A. Paasinen, L. C. Anderson & E. Hölttä: Ornithine decarboxylase activity is critical for cell transformation. *Nature* 360, 355-358 (1992)

31. Moshier J. A., J. Dosescu, M. Skunca & G. D. Luk: Transformation of NIH/3T3 cells by ornithine decarboxylase overexpression. *Cancer Res* 53, 2618-2622 (1993)

32. Packham G. & J. L. Cleveland: Ornithine decarboxylase is a mediator of c-Myc-induced apoptosis. *Mol Cell Biol* 14, 5741-5747 (1994)

33. Packham G., C. W. Porter & J. L. Cleveland: c-Myc induces apoptosis and cell cycle progression by separable, yet overlapping, pathways. *Oncogene* 13, 461-469 (1996)

34. Bouchard C., P. Staller & M. Eilers: Control of cell proliferation by Myc. *Trends Cell Biol* (1998) In press

35. Roy A. L., C. Carruthers, T. Gutjahr & R. G. Roeder: Direct role for Myc in transcription initiation mediated by interactions with TFII-I. *Nature* 365, 359-361 (1993)

36. Peukert K., P. Staller, A. Schneider, G. Carmichael, F. Hanel & M. Eilers: An alternative pathway for gene regulation by myc. *EMBO J* 16, 5672-5686 (1997)

37. Packham G. & J. L. Cleveland: c-Myc and apoptosis. *Biochim Biophys Acta* 1242, 11-28 (1995)

38. Pardee A. B.: G1 events and regulation of cell proliferation. *Science* 246, 603-608 (1989)

39. Zetterberg A., O. Larsson & K. G. Wiman: What is the restriction point? *Curr Opin Cell Biol* 7, 835-842 (1995)

40. Sherr C. J.: D-type cyclins. *Trends Biochem Sci* 20, 187-190 (1995)

41. Weinberg R. A.: The retinoblastoma protein and cell cycle control. *Cell* 81, 323-330 (1995)

42. Sherr C. J.: G1 phase progression: cycling on cue. *Cell* 79, 551-555 (1994)

43. Sherr C. J.: Mammalian G1 cyclins. *Cell* 73, 1059-1065 (1993)

44. Beijersbergen R. L., L. Carlee, R. M. Kerkhoven & R. Bernards: Regulation of the retinoblastoma protein-related p107 by G1 cyclin complexes. *Genes Dev* 9, 1340-1353 (1995)

45. Xiao Z.-X., D. Ginsberg, M. Ewen & D. M. Livingston: Regulation of the Retinoblastoma protein-related protein p107 by G1 cyclin-associated kinases. *Proc Natl Acad Sci USA* 93, 4633-4637 (1996)

46. Mayol X., J. Garriga & X. Grana: Cell cycle-dependent phosphorylation of the retinoblastoma-related protein p130. *Oncogene* 11, 801-808 (1995)

47. Beijersbergen R. L. & R. Bernards: Cell cycle regulation by the Retinoblastoma family of growth inhibitory proteins. *Biochim Biophys Acta* 1287, 103-120 (1996)

48. Mayol X. & X. Graña: The p130 pocket protein: keeping order at cell cycle exit/re-entrance transitions. Front Biosci 3, 11-24 (1998)

49. Müller R.: Transcriptional regulation during the mammalian cell cycle. Trends Genet 11, 173-178 (1995)

50. Weinberg R. A.: E2F and cell proliferation: a world turned upside down. *Cell* 85, 457-459 (1996)

51. Cobrinik D.: Regulatory interactions among E2Fs and cell cycle control proteins. *Curr Top Microbiol Immunol* 208, 31-61 (1996)

52. Krek W., D. M. Livingston & S. Shirodkar: Binding to DNA and the retinoblastoma gene product promoted by complex formation of different E2F family members. *Science* 262, 1557-1560 (1993)

53. Bandara L., V. M. Buck, M. Zamanian, L. H. Johnston and & N. LaThangue: Functional synergy between DP-1 and E2F-1 in the cell cycle regulating transcription factor DRTF1/E2F. *EMBO J* 12, 4317-4324 (1993)

54. Helin K., C. L. Wu, A. R. Fattaey, J. A. Lees, B. D. Dynlacht, C. Ngwu & E. Harlow: Heterodimerization of the transcription factors E2F-1 and DP-1 leads to cooperative trans-activation. *Genes Dev* 7, 1850-1861 (1993)

55. Bernards R.: E2F: a nodal point in cell cycle regulation. *Biochim Biophys Acta* 1333, M33-M40 (1997)

56. Morgan D. O.: Principles of CDK regulation. *Nature* 374, 131-134 (1995)

57. Sherr C. J. & J. M. Roberts: Inhibitors of mammalian G1 cyclin-dependent kinases. *Genes Dev* 9, 1149-1163 (1995)

58. LaBaer J., M. D. Garrett, L. F. Stevenson, J. M. Slingerland, C. Sandhu, H. S. Chou, A. Fattaey & E. Harlow: New functional activities for the p21 family of CDK inhibitors. *Genes Dev* 11, 847-862 (1997)

59. Harper J. W. & S. J. Elledge: Cdk inhibitors in development and cancer. *Curr Opin Genet Dev* 6, 56-64 (1996)

60. Lukas J., H. Muller, J. Bartkova, D. Spitkovsky, A. A. Kjerulff, P. Jansen-Durr, M. Strauss & J. Bartek: DNA tumor virus oncoproteins and retinoblastoma gene mutations share the ability to relieve the cell's requirement for cyclin D1 function in G1. *J Cell Biol* 125, 625-638 (1994)

61. Lukas J., J. Bartkova, M. Rohde, M. Strauss & J. Bartek: Cyclin D1 is dispensable for G1 control in retinoblastoma gene-deficient cells independently of cdk4 activity. *Mol Cell Biol* 15, 2600-2611 (1995a)

62. Lukas J., D. Parry, L. Aagaard, D. J. Mann, J. Bartkova, M. Strauss, G. Peters & J. Bartek: Retinoblastoma-protein-dependent cell-cycle inhibition by the tumour suppressor p16. *Nature* 375, 503-506 (1995b)

63. Koh J., G. H. Enders, B. D. Dynlacht & E. Harlow: Tumour-derived p16 alleles encoding proteins defective in cell-cycle inhibition. *Nature* 375, 506-510 (1995)

64. Medema R. H., R. E. Herrera, F. Lam & R. A. Weinberg: Growth suppression by p16ink4 requires functional retinoblastoma protein. *Proc Natl Acad Sci U S A* 92, 6289-6293 (1995)

65. DeGregori J., G. Leone, K. Ohtani, A. Miron & J. R. Nevins: E2F-1 accumulation bypasses a G1 arrest resulting

from the inhibition of G1 cyclin-dependent kinase activity. *Genes Dev* 9, 2873-2887 (1995)

66. Mann D. J. & N. C. Jones: E2F-1 but not E2F-4 can overcome p16-induced G1 cell-cycle arrest. *Curr Biol* 6, 474-483 (1996)

67. Lukas J., B. Otzen Petersen, K. Holm, J. Bartek & K. Helin: Deregulated expression of E2F family members induces S-phase entry and overcomes p16INK4A-mediated growth suppression. *Mol Cell Biol* 16, 1047-1057 (1996)

68. Resnitzky D. & S. I. Reed: Different roles for cyclins D1 and E in regulation of the G1-to-S transition. *Mol Cell Biol* 15, 3463-3469 (1995)

69. Hofmann F. & D. M. Livingston: Differential effects of cdk2 and cdk3 on the control of pRb and E2Ff function during G(1) exit. *Genes Dev* 10, 851-861 (1996)

70. Ohtsubo M., A. M. Theodoras, J. Schumacher, J. M. Roberts & M. Pagano: Human cyclin E, a nuclear protein essential for the G1-to-S phase transition. *Mol Cell Biol* 15, 2612-2624 (1995)

71. Lukas J., T. Herzinger, K. Hansen, M. Moroni, D. Resnitzky, K. Helin, S. Reed & J. Bartek: Cyclin E-induced S phase without activation of the pRb/E2F pathway. *Genes Dev* 11, 1479-1492 (1997)

72. Alevizopoulos K., J. Vlach, S. Hennecke & B. Amati: Cyclin E and c-Myc promote cell proliferation in the presence of p16INK4a and of hypophosphorylated Retinoblastoma-family proteins. *EMBO J* 16, 5322-5333 (1997)

73. Leng X. H., L. Connell-Crowley, D. Goodrich & J. W. Harper: S-phase entry upon ectopic expression of G1 cyclin-dependent kinases in the absence of retinoblastoma protein phosphorylation. *Curr Biol* 7, 709-712 (1997)

74. Ohtani K., J. DeGregori & J. R. Nevins: Regulation of the cyclin E gene by transcription factor E2F1. Proc Nal Acad Sci USA 92, 12146-12150 (1995)

75. Geng Y., E. N. Eaton, M. Picon, J. M. Roberts, A. S. Lundberg, A. Gifford, C. Sardet & R. A. Weinberg: Regulation of cyclin E transcription by E2Fs and retinoblastoma protein. *Oncogene* 12, 1173-1180 (1996)

76. Botz J., K. Zerfass-Thome, D. Spitkovsky, H. Delius, B. Vogt, M. Eilers, A. Hatzigeorgiou & P. Jansen-Dürr: Cell cycle regulation of the murine cyclin E gene depends on an E2F binding site in the promoter. *Mol Cell Biol* 16, 3401-3409 (1996)

77. Herrera R. E., V. P. Sah, B. O. Williams, T. P. Makela, R. A. Weinberg & J. Tyler: Altered cell cycle kinetics, gene expression, and G1 restriction point regulation in rb-deficient fibroblasts. *Mol Cell Biol* 16, 2402-2407 (1996)

78. Hurford R. J., D. Cobrinik, M. Lee & N. Dyson: pRB and p107/p130 are required for the regulated expression of different sets of E2F responsive genes. *Genes Dev* 11, 1447-1463 (1997)

79. DeGregori J., T. Kowalik & J. R. Nevins: Cellular targets for activation by the E2F1 transcription factor include DNA synthesis- and G1/S-regulatory genes. *Mol Cell Biol* 15, 4215-4224 (1995)

80. Soucek T., O. Pusch, E. Hengstschlager-Ottnad, P. D. Adams & M. Hengstschlager: Deregulated expression of E2f-1 induces cyclin A- and E-associated kinase activities independently from cell cycle position. *Oncogene* 14, 2251-2257 (1997)

81. Bartkova J., J. Lukas, M. Strauss & J. Bartek: Cyclin D1 oncoprotein aberrantly accumulates in malignancies of diverse histogenesis. *Oncogene* 10, 775-779 (1995)

82. Lukas J., L. Aagaard, M. Strauss & J. Bartek: Oncogenic aberrations of p16INK4/CDKN2 and cyclin D1 cooperate to deregulate G1 control. *Cancer Res* 55, 4818-4823 (1995)

83. Hall M. & G. Peters: Genetic alterations of cyclins, cyclin-dependent kinases, and cdk inhibitors in human cancer. Adv *Cancer Res* 68, 67-108 (1996)

84. Strauss M., J. Lukas & J. Bartek: Unrestricted cell cycling and cancer. *Nature Med* 1, 1245-1246 (1995)

85. Serrano M., H. Lee, L. Chin, C. Cordon-Cardo, D. Beach & R. A. DePinho: Role of the INK4a locus in tumor suppression and cell mortality. *Cell* 85, 27-37 (1996)

86. Quelle D. E., F. Zindy, R. A. Ashmun & C. J. Sherr: Alternative reading frames of the INK4a tumor suppressor gene encode two unrelated proteins capable of inducing cell cycle arrest. *Cell* 83, 993-1000 (1995)

87. Kamijo T., F. Zindy, M. F. Roussel, D. E. Quelle, J. R. Downing, R. A. Ashmun, G. Grosveld & C. J. Sherr: Tumor suppression at the mouse ink4a locus mediated by the alternative reading frame product p19(ARF). *Cell* 91, 649-659 (1997)

88. Vlach J., S. Hennecke, K. Alevizopoulos, D. Conti & B. Amati: Growth arrest by the cyclin-dependent kinase inhibitor p27Kip1 is abrogated by c-Myc. *EMBO J*. 15, 6595-6604 (1996)

89. Polyak K., J. Y. Kato, M. J. Solomon, C. J. Sherr, J. Massague, J. M. Roberts & A. Koff: p27Kip1, a cyclin-Cdk inhibitor, links transforming growth factor-beta and contact inhibition to cell cycle arrest. *Genes Dev* 8, 9-22 (1994)

90. Reynisdottir I., K. Polyak, A. Iavarone & J. Massague: Kip/Cip and Ink4 Cdk inhibitors cooperate to induce cell cycle arrest in response to TGF-beta. *Genes Dev* 9, 1831-1845 (1995)

91. Poon R. Y. C., H. Toyoshima & T. Hunter: Redistribution of the CDK inhibitor p27 between different cyclin-CDK complexes in the mouse fibroblast cell cycle and

in cells arrested with lovastatin and ultraviolet irradiation. Mol Biol *Cell* 6, 1197-1213 (1995)

92. Soos T. J., H. Kiyokawa, J. S. Yan, M. S. Rubin, A. Giordano, A. DeBlasio, S. Bottega, B. Wong, J. Mendelsohn & A. Koff: Formation of p27-CDK complexes during the human mitotic cell cycle. *Cell Growth Diff* 7, 135-146 (1996)

93. Rudolph B., R. Saffrich, J. Zwicker, B. Henglein, R. Müller, W. Ansorge & M. Eilers: Activation of cyclin-dependent kinases by Myc mediates induction of cyclin A, but not apoptosis. *EMBO J* 15, 3065-3076 (1996)

94. Fornace A. J., J. Jackman, M. C. Hollander, B. Hoffman-Liebermann & D. A. Liebermann: Genotoxic-stress-response genes and growth-arrest genes. gadd, MyD, and other genes induced by treatments eliciting growth arrest. Ann N Y Acad Sci 663, 139-53 (1992)

95. Zhan Q., K. A. Lord, I. J. Alamo, M. C. Hollander, F. Carrier, D. Ron, K. W. Kohn, B. Hoffman, D. A. Liebermann & A. J. Fornace: The gadd and MyD genes define a novel set of mammalian genes encoding acidic proteins that synergistically suppress cell growth. *Mol Cell Biol* 14, 2361-2371 (1994)

96. Vairapandi M., A. G. Balliet, A. J. J. Fornace, B. Hoffman & D. A. Liebermann: The differentiation primary response gene MyD118, related to GADD45, encodes for a nuclear protein which interacts with PCNA and p21WAF1/CIP1. *Oncogene* 12, 2579-2594 (1996)

97. Zhan Q., W. El-Deiry, I. Bae, I. Alamo, M. B. Kastan, B. Vogelstein & A. J. J. Fornace: Int J Oncol 6, 937-946 (1995)

98. Marhin W. W., S. J. Chen, L. M. Facchini, A. J. Fornace & L. Z. Penn: Myc represses the growth arrest gene gadd45. *Oncogene* 14, 2825-2834 (1997)

99. Steiner P., A. Philipp, J. Lukas, D. Godden-Kent, M. Pagano, S. Mittnacht, J. Bartek & M. Eilers: Identification of a Myc-dependent step during the formation of active G1 cyclin-cdk complexes. *EMBO J* 14, 4814-4826 (1995)

100. Jansen-Dürr P., A. Meichle, P. Steiner, M. Pagano, K. Finke, J. Botz, J. Wessbecher, G. Draetta & M. Eilers: Differential modulation of cyclin gene expression by MYC. *Proc Natl Acad Sci USA* 90, 3685-3689 (1993)

101. Perez-Roger I., D. L. C. Solomon, A. Sewing & H. Land: Myc activation of cyclin E/CDK2 kinase involves induction of cyclin E gene transcription and inhibition of p27Kip1 binding to newly formed complexes. *Oncogene* 14, 2373-2381 (1997)

102. Müller D., C. Bouchard, B. Rudolph, P. Steiner, I. Stuckmann, R. Saffrich, W. Ansorge, W. Huttner & M. Eilers: Cdk2-dependent phosphorylation of p27 facilitates its myc-induced release from cyclin E/cdk2 complexes. *Oncogene* 15, 2561-2576 (1997)

103. Pagano M., S. W. Tam, A. M. Theodoras, R. P. Beer, S. G. Del, V. Chau, P. R. Yew, G. F. Draetta & M. Rolfe: Role of the ubiquitin-proteasome pathway in regulating abundance of the cyclin-dependent kinase inhibitor p27. *Science* 269, 682-685 (1995)

104. Sheaff R. J., M. Groudine, M. Gordon, J. M. Roberts & B. E. Clurman: Cyclin E-CDK2 is a regulator of p27Kip1. *Genes Dev* 11, 1464-1478 (1997)

105. Vlach J., S. Hennecke & B. Amati: Phosphorylation-dependent degradation of the cyclin-dependent kinase inhibitor p27Kip1. *EMBO J* 16, 5334-5344 (1997)

106. Pusch O., G. Bernaschek, M. Eilers & M. Hengstschlager: Activation of c-Myc uncouples DNA replication from activation of G1-cyclin-dependent kinases. *Oncogene* 15, 649-656 (1997)

107. Hoffmann I., G. Draetta & E. Karsenti: Activation of the phosphatase activity of human cdc25A by a cdk2-cyclin E dependent phosphorylation at the G1/S transition. *EMBO J* 13, 4302-4310 (1994)

108. Saha P., Q. Eichbaum, E. D. Silberman, B. J. Mayer & A. Dutta: p21CIP1 and Cdc25A: competition between an inhibitor and an activator of cyclin-dependent kinases. *Mol Cell Biol* 17, 4338-4345 (1997)

109. Iavarone A. & J. Massague: Repression of the CDK activator Cdc25A and cell-cycle arrest by cytokine TGF-beta in cells lacking the CDK inhibitor p15. *Nature* 387, 417-422 (1997)

110. Berns K., E. M. Hijmans & R. Bernards: Repression of c-myc responsive genes in cycling cells causes G1 arrest through reduction of cyclin E-cdk2 kinase activity. *Oncogene* 15, 1347-1356 (1997)

111. Karn J., J. V. Watson, A. D. Lowe, S. M. Green & W. Vedeckis: Regulation of cell cycle duration by c-myc levels. *Oncogene* 4, 773-787 (1989)

112. Duronio R. J., P. H. O'Farrell, J. E. Xie, A. Brook & N. Dyson: The transcription factor E2F is required for S phase during Drosophila embryogenesis. *Genes Dev* 9, 1445-1455 (1995)

113. Duronio R. J. & P. H. O'Farrell: Developmental control of the G1 to S transition in Drosophila: cyclin E is a limiting downstream target of E2F. *Genes Dev* 9, 1456-1468 (1995)

114. Duronio R. J., A. Brook, N. Dyson & P. H. O'Farrel: E2F-induced S-phase entry requires cyclin E. *Genes Dev* 10, 2505-2513 (1996)

115. Leone G., J. DeGregori, R. Sears, L. Jakoi & J. R. Nevins: Myc and Ras collaborate in inducing accumulation of active cyclin E/Cdk2 and E2F. *Nature* 387, 422-426 (1997)

116. Sears R., K. Ohtani & J. R. Nevins: Identification of positively and negatively acting elements regulating

expression of the E2F2 gene in response to cell growth signals. *Mol Cell Biol* 17, 5227-5235 (1997)

117. Reynisdottir I. & J. Massague: The subcellular locations of p15(Ink4b) and p27(Kip1) coordinate their inhibitory interactions with cdk4 and cdk2. *Genes Dev* 11, 492-503 (1997)

118. Hannon G. J. & D. Beach: p15 INK4B is a potential effector of TGF-beta-induced growth arrest. *Nature* 371, 257-261 (1994)

119. Pietenpol J. A., R. W. Stein, E. Moran, P. Yaciuk, R. Schlegel, R. M. Lyons, M. R. Pittelkow, K. Munger, P. M. Howley & H. L. Moses: TGF-beta 1 inhibition of c-myc transcription and growth in keratinocytes is abrogated by viral transforming proteins with pRB binding domains. *Cell* 61, 777-785 (1990)

120. Matsushime H., M. F. Roussel, R. A. Ashmun & C. J. Sherr: Colony-stimulating factor 1 regulates novel cyclins during the G1 phase of the cell cycle. *Cell* 65, 701-713 (1991)

121. Daksis J. I., R. Y. Lu, L. M. Facchini, W. W. Marhin & L. J. Penn: Myc induces cyclin D1 expression in the absence of de novo protein synthesis and links mitogen-stimulated signal transduction to the cell cycle. *Oncogene* 9, 3635-3645 (1994)

122. Solomon D. L., A. Philipp, H. Land & M. Eilers: Expression of cyclin D1 mRNA is not upregulated by Myc in rat fibroblasts. *Oncogene* 11, 1893-1897 (1995)

123. Philipp A., A. Schneider, I. Vasrik, K. Finke, Y. Xiong, D. Beach, K. Alitalo & M. Eilers: Repression of cyclin D1: a novel function of MYC. *Mol Cell Biol* 14, 4032-4043 (1994)

124. Marhin W. W., Y. J. Hei, S. Chen, Z. Jiang, B. L. Gallie, R. A. Phillips & L. Z. Penn: Loss of Rb and Myc activation co-operate to suppress cyclin D1 and contribute to transformation. *Oncogene* 12, 43-52 (1996)

125. Rosenwald I. B., D. B. Rhoads, L. D. Callanan, K. J. Isselbacher & E. V. Schmidt: Increased expression of eukaryotic translation initiation factors eIF-4E and eIF-2 alpha in response to growth induction by c-myc. *Proc Natl Acad Sci U S A* 90, 6175-6178 (1993)

126. Jones R. M., J. Branda, K. A. Johnston, M. Polymenis,
M. Gadd, A. Rustgi, L. Callanan & E. V. Schmidt: An essential E box in the promoter of the gene encoding the mRNA cap-binding protein (eukaryotic initiation factor 4E) is a target for activation by c-myc. *Mol Cell Biol* 16, 4754-4764 (1996)

127. Rosenwald I. B., K. A. Lazaris, N. Sonenberg & E. V. Schmidt: Elevated levels of cyclin D1 protein in response to increased expression of eukaryotic initiation factor 4E. *Mol Cell Biol* 13, 7358-7363 (1993)

128. Roussel M. F., J. L. Cleveland, S. A. Shurtleff & C. J. Sherr: Myc rescue of a mutant CSF-1 receptor impaired in mitogenic signalling. *Nature* 353, 361-363 (1991)

129. Roussel M. F., A. M. Theodoras, M. Pagano & C. J. Sherr: Rescue of defective mitogenic signaling by D-type cyclins. *Proc Natl Acad Sci U S A* 92, 6837-6841 (1995)

130. Hiebert S. W., M. Lipp & J. R. Nevins: E1A-dependent trans-activation of the human MYC promoter is mediated by the E2F factor. *Proc Natl Acad Sci U S A* 86, 3594-8 (1989)

131. Thalmeier K., H. Synovzik, R. Mertz, E. L. Winnacker & M. Lipp: Nuclear factor E2F mediates basic transcription and trans-activation by E1a of the human MYC promoter. *Genes Dev* 3, 527-36 (1989)

132. Mudryj M., S. W. Hiebert & J. R. Nevins: A role for the adenovirus inducible E2F transcription factor in a proliferation dependent signal transduction pathway. *EMBO J* 9, 2179-2184 (1990)

133. Oswald F., H. Lovec, T. Moroy & M. Lipp: E2F-dependent regulation of human MYC: trans-activation by cyclins D1 and A overrides tumour suppressor protein functions. *Oncogene* 9, 2029-2036 (1994)

134. Shibuya H., M. Yoneyama, T. J. Ninomiya, K. Matsumoto & T. Taniguchi: IL-2 and EGF receptors stimulate the hematopoietic cell cycle via different signaling pathways: demonstration of a novel role for c-myc. *Cell* 70, 57-67 (1992)

135. Langer S. J., D. M. Bortner, M. F. Roussel, C. J. Sherr & M. C. Ostrowski: Mitogenic signaling by colony-stimulating factor 1 and ras is suppressed by the ets-2 DNA-binding domain and restored by myc overexpression. *Mol Cell Biol* 12, 5355-62 (1992)

136. Roussel M. F., J. N. Davis, J. L. Cleveland, J. Ghysdael & S. W. Hiebert: Dual control of myc expression through a single DNA binding site targeted by ets family proteins and E2F-1. *Oncogene* 9, 405-15 (1994)

137. Barone M. V. & S. A. Courtneidge: Myc but not Fos rescue of PDGF signalling block caused by kinase-inactive Src. *Nature* 378, 509-512 (1995)

138. Zou X., S. Rudchenko, K. Wong & K. Calame: Induction of c-myc transcription by the v-Abl tyrosine kinase requires Ras, Raf1, and cyclin-dependent kinases. *Genes Dev* 11, 654-662 (1997)

139. Wong K. K., X. Zou, K. T. Merrell, A. J. Patel, K. B. Marcu, S. Chellappan & K. Calame: v-Abl activates c-myc transcription through the E2F site. *Mol Cell Biol* 15, 6535-6544 (1995)

140. Hermeking H., D. A. Wolf, F. Kohlhuber, A. Dickmanns, M. Billaud, E. Fanning & D. Eick: Role of c-myc in simian virus 40 large tumor antigen-induced DNA synthesis in quiescent 3T3-L1 mouse fibroblasts. *Proc Natl*

Acad Sci U S A 91, 10412-10416 (1994)

141. Gu W., K. Bhatia, I. Magrath, C. Dang & R. Dalla-Favera: Binding and suppression of the Myc transcriptional activation domain by p107. *Science* 264, 251-254 (1994)

142. Beijersbergen R. L., E. M. Hijmans, L. Zhu & R. Bernards: Interaction of c-Myc with the pRb-related protein p107 results in inhibition of c-Myc-mediated transactivation. *EMBO J* 13, 4080-4086 (1994)

143. Hoang A. T., B. Lutterbach, B. C. Lewis, T. Yano, T. Y. Chou, J. F. Barrett, M. Raffeld, S. R. Hann & C. V. Dang: A link between increased transforming activity of lymphoma-derived MYC mutant alleles, their defective regulation by p107, and altered phosphorylation of the c-Myc transactivation domain. *Mol Cell Biol* 15, 4031-4042 (1995)

144. Smith-Sorensen B., E. M. Hijmans, R. L. Beijersbergen & R. Bernards: Functional analysis of burkitts lymphoma mutant c-myc proteins. J Biol Chem 271, 5513-5518 (1996)

145. Haas K., P. Staller, C. Geisen, J. Bartek, M. Eilers & T. Moroy: Mutual requirement of CDK4 and Myc in malignant transformation: evidence for cyclin D1/CDK4 and p16INK4A as upstream regulators of Myc. *Oncogene* 15, 179-192 (1997)

146. Qin X.-Q., D. M. Livingston, M. Ewen, W. R. Sellers, Z. Arany & W. G. Kaelin: The transcription factor E2F-1 is a downstream target of RB action. *Mol Cell Biol* 15, 742-755 (1995)

147. Zhu L., S. van den Heuvel, K. Helin, A. Fattaey, M. Ewen, D. M. Livingston, N. Dyson & E. Harlow: Inhibition of cell proliferation by p107, a relative of the retinoblastoma protein. *Genes Dev* 7, 1111-1125 (1993)

148. Mateyak M. K., A. J. Obaya, S. Adachi & J. M. Sedivy: Phenotypes of c-myc-deficient rat fibroblasts isolated by targeted homologous recombination. Cell Growth Differ 8, 1039-1048 (1997)

149. Ittmann M., J. Ali, A. Greco & C. Basilico: The gene complementing a temperature-sensitive cell cycle mutant of BHK cells is the human homologue of the yeast RPC53 gene, which encodes a subunit of RNA polymerase C (III). *Cell* Growth Differ 4, 503-511 (1993)

150. Jackson A. J., M. Ittmann & B. F. Pugh: The BN51 protein is a polymerase (Pol)-specific subunit of RNA Pol III which reveals a link between Pol III transcription and pre-rRNA processing. *Mol Cell Biol* 15, 94-101 (1995)

151. Ghisolfi-Nieto L., G. Joseph, F. Puvion-Dutilleul, F. Amalric & P. Bouvet: Nucleolin is a sequence-specific RNA-binding protein: characterization of targets on pre-ribosomal RNA. J Mol Biol 260, 34-53 (1996)

152. Ittmann M., A. Greco & C. Basilico: Isolation of the human gene that complements a temperature-sensitive cell cycle mutation in BHK cells. *Mol Cell Biol* 7, 3386-3393 (1987)

153. Cavanaugh A. H., W. M. Hempel, L. J. Taylor, V. Rogalsky, G. Todorov & L. I. Rothblum: Activity of RNA polymerase I transcription factor UBF blocked by Rb gene product. *Nature* 374, 177-180 (1995)

154. White R. J., D. Trouche, K. Martin, S. P. Jackson & T. Kouzarides: Repression of RNA polymerase III transcription by the retinoblastoma protein. *Nature* 382, 88-90 (1996)

155. Chu W. M., Z. Wang, R. G. Roeder & C. W. Schmid: RNA polymerase III transcription repressed by Rb through its interactions with TFIIIB and TFIIIC2. J Biol Chem 272, 14755-14761 (1997)

156. Larminie C. G., C. A. Cairns, R. Mital, K. Martin, T. Kouzarides, S. P. Jackson & R. J. White: Mechanistic analysis of RNA polymerase III regulation by the retinoblastoma protein. *EMBO J* 16, 2061-2071 (1997)

157. Voit R., K. Schafer & I. Grummt: Mechanism of repression of RNA polymerase I transcription by the retinoblastoma protein. *Mol Cell Biol* 17, 4230-4237 (1997)

158. Herwig S. & M. Strauss: The retinoblastoma protein: a master regulator of cell cycle, differentiation and apoptosis. *Eur J Biochem* 246, 581-601 (1997)

159. Downward J.: Cell cycle: routine role for Ras. *Curr Biol* 7, (1997)

160. Marshall C. J.: Ras effectors. *Curr Opin Cell Biol* 8, 197-204 (1996)

161. Pronk G. J. & J. L. Bos: The role of p21ras in receptor tyrosine kinase signalling. *Biochim Biophys Acta* 1198, 131-147 (1994)

162. Dobrowolski S., M. Harter & D. W. Stacey: Cellular ras activity is required for passage through multiple points of the G0/G1 phase in BALB/c 3T3 cells. *Mol Cell Biol* 14, 5441-5449 (1994)

163. Peeper D. S., T. M. Upton, M. H. Ladha, E. Neuman, J. Zalvide, R. Bernards, J. A. Decaprio & M. E. Ewen: Ras signalling linked to the cell-cycle machinery by the retinoblastoma protein. *Nature* 386, 177-181 (1997)

164. Mittnacht S., H. Paterson, M. F. Olson & C. J. Marshall: Ras signalling is required for inactivation of the tumour suppressor pRb cell-cycle control protein. *Curr Biol* 7, 219-221 (1997)

165. Aktas H., H. Cai & G. M. Cooper: Ras links growth factor signaling to the cell cycle machinery via regulation of cyclin D1 and the Cdk inhibitor p27KIP1. *Mol Cell Biol* 17, 3850-3857 (1997)

166. Sewing A., B. Wiseman, A. C. Lloyd & H. Land: High-intensity Raf signal causes cell cycle arrest mediated by p21Cip1. *Mol Cell Biol* 17, 5588-5597 (1997)

167. Woods D., D. Parry, H. Cherwinski, E. Bosch, E. Lees & M. McMahon: Raf-induced proliferation or cell cycle arrest is determined by the level of Raf activity with arrest mediated by p21Cip1. *Mol Cell Biol* 17, 5598-55611 (1997)

168. Kawada M., S. Yamagoe, Y. Murakami, K. Suzuki, S. Mizuno & Y. Uehara: Induction of p27Kip1 degradation and anchorage independence by Ras through the MAP kinase signaling pathway. *Oncogene* 15, 629-637 (1997)

169. Galaktionov K., C. Jessus & D. Beach: Raf1 interaction with Cdc25 phosphatase ties mitogenic signal transduction to cell cycle activation. *Genes Dev* 9, 1046-1058 (1995)

170. Connell-Crowley L., S. J. Elledge & J. W. Harper: G1 cyclin-dependent kinases are sufficient to initiate DNA synthesis in quiescent human fibroblasts. *Curr Biol* 8, 65-68 (1998)

171. Flynn A. & C. G. Proud: The role of eIF4 in cell proliferation. *Cancer Surv* 27, 293-310 (1996)

172. Graff J. R., E. R. Boghaert, B. A. De, D. L. Tudor, C. C. Zimmer, S. K. Chan & S. G. Zimmer: Reduction of translation initiation factor 4E decreases the malignancy of ras-transformed cloned rat embryo fibroblasts. Int J Cancer 60, 255-263 (1995)

173. Rinker-Schaeffer C. W., J. R. Graff, A. De Benedetti, S. G. Zimmer & R. E. Rhoads: Decreasing the level of translation initiation factor 4E with antisense RNA causes reversal of ras-mediated transformation and tumorigenesis of cloned rat embryo fibroblasts. *Int J Cancer* 55, 841-847 (1993)

174. Polunovsky V. A., I. B. Rosenwald, A. T. Tan, J. White, L. Chiang, N. Sonenberg & P. B. Bitterman: Translational control of programmed cell death: eukaryotic translation initiation factor 4E blocks apoptosis in growth-factor-restricted fibroblasts with physiologically expressed or deregulated Myc. *Mol Cell Biol* 16, 6573-6581 (1996)

175. Land H., A. C. Chen, J. P. Morgenstern, L. F. Parada & R. A. Weinberg: Behavior of myc and ras *Oncogene*s in transformation of rat embryo fibroblasts. *Mol Cell Biol* 6, 1917-1925 (1986)

176. Land H., L. F. Parada & R. A. Weinberg: Cellular *Oncogene*s and multistep carcinogenesis. *Science* 222, 771-778 (1983)

177. Sinn E., W. Muller, P. Pattengale, I. Tepler, R. Wallace & P. Leder: Coexpression of MMTV/v-Ha-ras and MMTV/c-myc genes in transgenic mice: synergistic action of *Oncogene*s *in vivo*. *Cell* 49, 465-475 (1987)

178. Sandgren E. P., C. J. Quaife, C. A. Pinkert, R. D. Palmiter & R. L. Brinster: *Oncogene*-induced liver neoplasia in transgenic mice. *Oncogene* 4, 715-24 (1989)

179. Langdon W. Y., A. W. Harris & S. Cory: Acceleration of B-lymphoid tumorigenesis in E mu-myc transgenic mice by v-H-ras and v-raf but not v-abl. *Oncogene* Res 4, 253-258 (1989)

180. Thompson T. C., J. Southgate, G. Kitchener & H. Land: Multistage carcinogenesis induced by ras and myc *Oncogene*s in a reconstituted organ. *Cell* 56, 917-930 (1989)

181. Haupt Y., A. W. Harris & J. M. Adams: Retroviral infection accelerates T lymphomagenesis in E mu-N-ras transgenic mice by activating c-myc or N-myc. *Oncogene* 7, 981-986 (1992)

182. Ruley H. E.: Transforming collaborations between ras and nuclear *Oncogene*s. Cancer Cells 2, 258-268 (1990)

183. Newbold R. F. & R. W. Overell: Fibroblast immortality is a prerequisite for transformation by EJ c-Ha-ras *Oncogene*. *Nature* 304, 648-651 (1983)

184. Franza B. J., K. Maruyama, J. I. Garrels & H. E. Ruley: *In vitro* establishment is not a sufficient prerequisite for transformation by activated ras *Oncogene*s. *Cell* 44, 409-418 (1986)

185. Ridley A. J., H. F. Paterson, M. Noble & H. Land: Ras-mediated cell cycle arrest is altered by nuclear *Oncogene*s to induce Schwann cell transformation. *EMBO J* 7, 1635-1645 (1988)

186. Lloyd A. C., F. Obermuller, S. Staddon, C. F. Barth, M. McMahon & H. Land: Cooperating *Oncogene*s converge to regulate cyclin/cdk complexes. *Genes Dev* 11, 663-677 (1997)

187. Serrano M., A. W. Lin, M. E. McCurrach, D. Beach & S. W. Lowe: Oncogenic ras provokes premature cell senescence associated with accumulation of p53 and p16INK4a. *Cell* 88, 593-602 (1997)

188. Kohl N. E. & H. E. Ruley: Role of c-myc in the transformation of REF52 cells by viral and cellular *Oncogene*s. *Oncogene* 2, 41-48 (1987)

189. Tsukada T., Y. Tomooka, S. Takai, Y. Ueda, S. Nishikawa, T. Yagi, T. Tokunaga, N. Takeda, Y. Suda, S. Abe, I. Matsuo, Y. Ikawa & S. Aizawa: Enhanced proliferative potential in culture of cells from p53-deficient mice. *Oncogene* 8, 3313-3322 (1993)

190. Harvey M., A. T. Sands, R. S. Weiss, M. E. Hegi, R. W. Wiseman, P. Pantazis, B. C. Giovanella, M. A. Tainsky, A. Bradley & L. A. Donehower: *In vitro* growth characteristics of embryo fibroblasts isolated from p53-deficient mice. *Oncogene* 8, 2457-2467 (1993)

191. Tanaka N., M. Ishihara, M. Kitagawa, H. Harada, T. Kimura, T. Matsuyama, M. S. Lamphier, S. Aizawa, T. W. Mak & T. Taniguchi: Cellular commitment to *Oncogene*-induced transformation or apoptosis is dependent on the transcription factor IRF-1. *Cell* 77, 829-839 (1994)

192. Donehower L. A., M. Harvey, B. L. Slagle, M. J. McArthur, C. A. J. Montgomery, J. S. Butel & A. Bradley:

Mice deficient for p53 are developmentally normal but susceptible to spontaneous tumours. *Nature* 356, 215-221 (1992)

193. Chin L., J. Pomerantz, D. Polsky, M. Jacobson, C. Cohen, C. Cordoncardo, J. W. Horner & R. A. Depinho: Cooperative effects of ink4a and ras in melanoma susceptibility *in vivo*. *Genes Dev* 11, 2822-2834 (1997)

194. Quelle D. E., M. Cheng, R. A. Ashmun & C. J. Sherr: Cancer-associated mutations at the INK4a locus cancel cell cycle arrest by p16INK4a but not by the alternative reading frame protein p19ARF. *Proc Natl Acad Sci U S A* 94, 669-673 (1997)

195. Deng C., P. Zhang, J. W. Harper, S. J. Elledge & P. Leder: Mice lacking p21CIP1/WAF1 undergo normal development, but are defective in G1 checkpoint control. *Cell* 82, 675-684 (1995)

196. Blyth K., A. Terry, M. O'Hara, E. W. Baxter, M. Campbell, M. Stewart, L. A. Donehower, D. E. Onions, J. C. Neil & E. R. Cameron: Synergy between a human c-myc transgene and p53 null genotype in murine thymic lymphomas: contrasting effects of homozygous and heterozygous p53 loss. *Oncogene* 10, 1717-1723 (1995)

197. Elson A., C. Deng, T. J. Campos, L. A. Donehower & P. Leder: The MMTV/c-myc transgene and p53 null alleles collaborate to induce T-cell lymphomas, but not mammary carcinomas in transgenic mice. *Oncogene* 11, 181-190 (1995)

198. Hsu B., M. C. Marin, A. k. el Naggar, L. C. Stephens, S. Brisbay & T. J. McDonnell: Evidence that c-myc mediated apoptosis does not require wild-type p53 during lymphomagenesis. *Oncogene* 11, 175-179 (1995)

199. Metz T., A. W. Harris & J. M. Adams: Absence of p53 allows direct immortalization of hematopoietic cells by the myc and raf *Oncogene*s. *Cell* 82, 29-36 (1995)

200. Hermeking H. & D. Eick: Mediation of c-Myc-induced apoptosis by p53. *Science* 265, 2091-2093 (1994)

201. Wagner A. J., J. M. Kokontis & N. Hay: Myc-mediated apoptosis requires wild-type p53 in a manner independent of cell cycle arrest and the ability of p53 to induce p21waf1/cip1. *Genes Dev* 8, 2817-2830 (1994)

202. Vaux D. L., S. Cory & J. M. Adams: Bcl-2 gene promotes haemopoietic cell survival and cooperates with c-myc to immortalize pre-B cells. *Nature* 335, 440-442 (1988)

203. Lovec H., A. Grzeschiczek, M. B. Kowalski & T. Moroy: Cyclin D1/bcl-1 cooperates with myc genes in the generation of B-cell lymphoma in transgenic mice. *EMBO J* 13, 3487-3495 (1994)

204. Bodrug S. E., B. J. Warner, M. L. Bath, G. J. Lindeman, A. W. Harris & J. M. Adams: Cyclin D1 transgene impedes lymphocyte maturation and collaborates in lymphomagenesis with the myc gene. *EMBO J* 13, 2124-2130 (1994)

205. Serrano M., L. E. Gomez, R. A. DePinho, D. Beach & S. D. Bar: Inhibition of ras-induced proliferation and cellular transformation by p16INK4. *Science* 267, 249-252 (1995)

206. Haas K., C. Johannes, C. Geisen, T. Schmidt, H. Karsunky, S. Blasskampmann, G. Obe & T. Moroy: Malignant transformation by cyclin E and ha-ras correlates with lower sensitivity towards induction of cell death but requires functional myc and Cdk4. *Oncogene* 15, 2615-2623 (1997)

207. Bortner D. M. & M. P. Rosenberg: Induction of mammary gland hyperplasia and carcinomas in transgenic mice expressing human cyclin E. *Mol Cell Biol* 17, 453-459 (1997)

208. Keyomarsi K., N. O'Leary, G. Molnar, E. Lees, H. J. Fingert & A. B. Pardee: Cyclin E, a potential prognostic marker for breast cancer. *Cancer Res* 54, 380-385 (1994)

209. Porter P. L., K. E. Malone, P. J. Heagerty, G. M. Alexander, L. A. Gatti, E. J. Firpo, J. R. Daling & J. M. Roberts: Expression of cell-cycle regulators p27(kip1) and cyclin E, alone and in combination, correlate with survival in young breast cancer patients. *Nature* Med 3, 222-225 (1997)

210. Gray-Bablin J., J. Zalvide, M. P. Fox, C. J. Knickerbocker, J. A. DeCaprio & K. Keyomarsi: Cyclin E, a redundant cyclin in breast cancer. *Proc Natl Acad Sci U S A* 93, 15215-15220 (1996)

211. Harper J. & S. Elledge: Cdk inhibitors in development and cancer. *Curr Opin Genet Dev* 6, 56-64 (1996)

212. Nakayama K., N. Ishida, M. Shirane, A. Inomata, T. Inoue, N. Shishido, I. Hori, D. Y. Loh & K. Nakayama: Mice lacking p27(kip1) display increased body size, multiple organ hyperplasia, retinal dysplasia, and pituitary tumors. *Cell* 85, 707-720 (1996)

213. Kiyokawa H., R. D. Kineman, K. O. Manovatodorova, V. C. Soares, E. S. Hoffman, M. Ono, D. Khanam, A. C. Hayday, L. A. Frohman & A. Koff: Enhanced growth of mice lacking the cyclin-dependent kinase inhibitor function of p27(kip1). *Cell* 85, 721-732 (1996)

214. Fero M. L., M. Rivkin, M. Tasch, P. Porter, C. E. Carow, E. Firpo, K. Polyak, L. H. Tsai, V. Broudy, R. M. Perlmutter, K. Kaushansky & J. M. Roberts: A syndrome of multiorgan hyperplasia with features of gigantism, tumorigenesis, and female sterility in p27(kip1)-deficient mice. *Cell* 85, 733-744 (1996)

215. Yan Y., J. Frisen, M. H. Lee, J. Massague & M. Barbacid: Ablation of the CDK inhibitor p57Kip2 results in increased apoptosis and delayed differentiation during mouse development. *Genes Dev* 11, 973-983 (1997)

216. Zhang P., N. Liégeois, C. Wong, M. Finegold, H. Hou, J. Thompson, A. Silverman, J. Harper, R. DePinho & S. Elledge: Altered cell differentiation and proliferation in mice

lacking p57KIP2 indicates a role in Beckwith-Wiedemann syndrome. *Nature* 387, 151-158 (1997)

217. Gao X., Y. Chen, N. Wu, D. Grignon, W. Sakr, A. Porter & K. Honn: Oncogenic mutations of the WAF1/CIP1 gene in primary prostate cancer. *Oncogene* 11, 1395-1398 (1995)

218. Malkowicz S., J. Tomaszewski, A. Linnenbach, T. Cangiano, Y. Maruta & T. McGarvey: Novel p21 mutations in superficial and invasive transitional cell carcinomas. *Oncogene* 13, 1831-1837 (1996)

219. Thompson J., K. Reese, M. DeBaun, E. Perlman & A. Feinberg: Reduced expression of the cyclin-dependent kinase inhibitor gene p57KIP2 in Wilms' tumor. *Cancer Res* 56, 5723-5727 (1996)

220. Catzavelos C., N. Bhattacharva, Y. C. Ung, J. A. Wilson, L. Roncari, C. Sandhu, P. Shaw, H. Yeger, I. Morava-Protzner, L. Kapusta, E. Franssen, K. I. Pritchard & J. M. Slingerland: Decreased levels of the cell-cycle inhibitor p27Kip1 protein: Prognostic implications in primary breast cancer. *Nature* Med 3, 227-230 (1997)

221. Loda M., B. Cukor, S. W. Tam, P. Lavin, M. Fiorentino, G. F. Draetta, J. M. Jessup & M. Pagano: Increased proteasome-dependent degradation of the cyclin-dependent kinase inhibitor p27 in agressive colorectal carcinomas. *Nature* Med 3, 231-234 (1997)

222. Xie J. W., M. Murone, S. M. Luoh, A. Ryan, Q. M. Gu, C. H. Zhang, J. M. Bonifas, C. W. Lam, M. Hynes, A. Goddard, A. Rosenthal, E. H. Epstein & F. J. Desauvage: Activating smoothened mutations in sporadic basal-cell carcinoma. *Nature* 391, 90-92 (1998)

223. Hunter T.: Oncoprotein networks. *Cell* 88, 333-346 (1997)

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