**G₁ Cyclin/Cyclin-dependent Kinase-coordinated Phosphorylation of Endogenous Pocket Proteins Differentially Regulates Their Interactions with E2F4 and E2F1 and Gene Expression***

Joaquim Calbó‡¶, Matilde Parreño‡, Elena Sotillo‡, Thomas Yong‡, Adela Mazo§, Judit Garriga‡, and Xavier Graña‡

From the ‡Fels Institute for Cancer Research and Molecular Biology and Department of Biochemistry, Temple University School of Medicine, Philadelphia, Pennsylvania 19140 and the §Department of Biochemistry and Molecular Biology, University of Barcelona, 08028 Barcelona, Spain

Mitogenic stimulation leads to activation of G₁ cyclin-dependent kinases (CDKs), which phosphorylate pocket proteins and trigger progression through the G₁/S transitions of the cell cycle. However, the individual role of G₁ cyclin-CDK complexes in the coordinated regulation of pocket proteins and their interaction with E2F family members is not fully understood. Here we report that individually or in concert cyclin D1-CDK and cyclin E-CDK complexes induce distinct and coordinated phosphorylation of endogenous pocket proteins, which also has distinct consequences in the regulation of pocket protein interactions with E2F4 and the expression of p107 and E2F1, both E2F-regulated genes. The up-regulation of these two proteins and the release of p130 and pRB from E2F4 complexes allows formation of E2F1 complexes not only with pRB but also with p130 and p107 as well as the formation of p107-E2F4 complexes. The formation of these complexes occurs in the presence of active cyclin D1-CDK and cyclin E-CDK complexes, indicating that whereas phosphorylation plays a role in the abrogation of certain pocket protein/E2F interactions, these same activities induce the formation of other complexes in the context of a cell expressing endogenous levels of pocket and E2F proteins. Of note, phosphorylated p130 “form 3,” which does not interact with E2F4, readily interacts with E2F1. Our data also demonstrate that ectopic overexpression of either cyclin is sufficient to induce mitogen-independent growth in human T98G and Rat-1 cells, although the effects of cyclin D1 require downstream activation of cyclin E-CDK2 activity. Interestingly, in T98G cells, cyclin D1 induces cell cycle progression more potently than cyclin E. This suggests that cyclin D1 activates pathways independently of cyclin E that ensure timely progression through the cell cycle.

---

*This work was supported in part by grants (to X. G.) including NIGMS, National Institutes of Health (NIH), Grant NIH-R29, GM54894; NIAID (NIH) Grant NIH R01, AI45450; NIAID (NIH) Career Development Award K02 AI01823; and W. W. Smith Grant A9802/9801. Facilities used for this work were supported in part by Shared Resources for Cancer Research Grant R24 (CA58261-01). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 15 U.S.C. Section 1734 solely to indicate this fact.¶Partially supported by fellowships from Direcció General de Investigación Científica y Técnica (Ministerio de Educación y Cultura, Spain).

†To whom correspondence should be addressed: Fels Institute for Cancer Research and Molecular Biology, Temple University School of Medicine, AHP Bldg., Rm. 308, 3307 N. Broad St., Philadelphia, PA 19140. Tel.: 215-707-7416; Fax: 215-707-5562; E-mail: xavier@unix.temple.edu.

---

G₁ cyclin-dependent kinases (CDKs)\(^1\) regulate progression through the G₀/G₁ transition and entry into the S-phase of the cell cycle following activation by mitogenic signaling pathways (1–5). G₁ CDKs phosphorylate the three members of the retinoblastoma family of pocket proteins, pRB, p107, and p130, resulting in cell cycle-dependent inactivation of their growth suppressor activities (6–13) (reviewed in Ref. 14).

Ectopic expression of cyclin D1 and cyclin E in primary or immortal, nontransformed mammalian fibroblasts shortens the G₁ phase of the cell cycle (15–17). The relatively modest effects of ectopic expression of G₁ cyclins in primary or immortal, nontransformed mammalian fibroblasts are probably due to a requirement for additional events to ensure full activation of these complexes. Whereas cyclins are limiting subunits for activation of their corresponding CDKs, full activation of cyclin-CDK complexes requires other events also dependent upon mitogenic stimulation (reviewed in Refs. 18–20). In agreement with this idea, microinjection of purified recombinant active cyclin D1-CDK4 or cyclin E-CDK2 complexes in human primary lung fibroblasts bypasses the requirement for mitogenic signaling (21). It has been suggested that cyclin D1-CDK effects trigger pRB inactivation and activation of E2F-dependent genes including cyclin E (21), whereas cyclin E-CDK2 effects might be independent of E2F (22, 23).

Initial studies suggested that pRB was the only substrate of D-type cyclin-CDKs required for a p16-mediated G₁ arrest (24–26). However, more recent studies demonstrated that, similar to the lack of pRB, lack of both p130 and p107 also prevents a p16-mediated G₁ arrest in primary mouse embryo fibroblasts, strongly suggesting that the three pocket proteins are important substrates for the cell cycle-regulatory functions of D-type cyclin-CDKs. Supporting this hypothesis further, it has been shown that both p130 and p107 are phosphorylated in vivo by D-type cyclin-CDK complexes at specific residues, which are not phosphorylated by cyclin E-CDK2 complexes (27, 28). Phosphorylation of these sites modulates the growth suppressor activities of p130 and p107. It is likely that two parallel pathways, one controlled by pRB and the other by p130/p107, regulate distinct downstream events required for G₁ progression into S phase. In agreement with this possibility, different sets of E2F-dependent genes are deregulated in mouse embryo fibroblasts lacking pRB and mouse embryo fibroblasts lacking both p130 and p107 (29).

---

The abbreviations used are: CDK, cyclin-dependent kinase; FBS, fetal bovine serum; CAPS, 3-(cyclohexylamino)propanesulfonic acid; PARP, poly(ADP-ribose) polymerase; CFSE, carboxymethyl fluorescein diacetate succinyl ester.
It is currently thought that pRB interacts with E2F1, E2F2, E2F3, and E2F4, whereas p130 and p107 interact with E2F4 but not with E2F1–3 (reviewed in Refs. 14 and 30). The unique ability of pRB to specifically interact with E2F1–3 supports the hypothesis that pRB controls a pathway, which is different from the pathway controlled by p130/p107.

Although it seems clear that cells will synthesize DNA when a certain threshold of cyclin D-CDK or cyclin E-CDK2 activity is induced in a cell, the concerted, as well as individual, effects of G1 cyclin-CDK activities on the coordinated phosphorylation of the three endogenous pocket proteins and the subsequent effects on E2F-dependent gene expression are not well understood. To address these questions in more detail, we have studied the downstream events induced by both cyclin D1 and cyclin E and their dependence on each other in human T98G and Rat-1 cells. Both cyclins are sufficient to induce mitogenic independent growth. The effects of both cyclins in endogenous pocket protein phosphorylation are clearly distinct. Phosphorylation of certain pocket proteins is sufficient to disrupt, at least partially, a subset of pocket protein-E2F complexes. However, because of the cell cycle-coordinated expression of endogenous members of the pocket protein and E2F families, new pocket protein-E2F complexes are formed even in the presence of active G1 CDKs. Surprisingly, we have found that both p130 and p107 specifically interact with E2F1 in a cell cycle-dependent manner. This work demonstrates that the interactions between pocket proteins and E2F family members are more complex than hereto anticipated and suggests cross-talk between these pathways.

EXPERIMENTAL PROCEDURES

Cell Culture and Cell Cycle Synchronization—T98G and Rat-1 cells were maintained in Dulbecco’s modified Eagle’s medium (Cellgro) supplemented with 10% fetal bovine serum (FBS) (Sigma) at 37 °C in a humidified atmosphere with 5% CO2. Cells were synchronized in G0 phase by contact inhibition followed by serum starvation. Briefly, cells were grown and kept overconfluent for 2 days. Cells were then trypsinized, counted, and seeded at 2 × 105 cells/plate in 100-mm dishes in MCDB-105 medium without FBS (Sigma). After 12 h, medium was removed, and fresh Dulbecco’s modified Eagle’s medium without FBS was added. Cells were kept for another 60 h before starting experiments.

Roscovitine was used to specifically inhibit CDK2 activity in cells infected with cyclin D1 adenoviruses. Infections were performed in the presence of either the CDK2/CDC2 inhibitor roscovitine (31) at a concentration of 25 μM, or vehicle (Me,SO).

For G1/M blockage and release experiments, serum-starved T98G cells restimulated with 10% FBS or infected with the indicated adenovirus for 22 h were incubated in the presence of 5% CO2 for an additional 22 h. At indicated time points, cells were collected and analyzed by flow cytometric and/or Western blot analysis.

Antibodies—Anti-p107 (sc-318), anti-p21 (sc-397), anti-p27 (sc-528), anti-cyclin A (sc-596), anti-cyclin E2 (sc-247 and sc-248), and anti-CDK2 (sc-163, anti-E2F1 (sc-193), and anti-E2F4 (sc-512) rabbit polyclonal antibodies and anti-cyclin D1 (sc-8906, and cyclin E (sc-251) mouse monoclonal antibodies were obtained from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Anti-p30 monoclonal antibody (R27020) was from Transduction Laboratories. Anti-p16 monoclonal antibody (G175–405) was from Pharmingen. Anti-pRB was a mix of monoclonal antibodies (X235, X256, X261, X291, X2105, X121, X1213, and X1455), which were provided by J. Harlow.

Adenovirus Production and Infection—Recombinant adenoviruses encoding cyclin D1 and cyclin E (Ad-Cyc D1 and Ad-Cyc E) were provided by J. Albrecht (33). Adenoviruses encoding p16 (Ad-p16) were provided by W. El-Deiry. Adenoviruses encoding enhanced green fluorescent protein (E2F3) and p107 (Ad-p107) were a gift of W. El-Deiry. Adenoviruses encoding p16, p130, and p107 specifically interact with E2F1 in a cell cycle-dependent manner. This work demonstrates that the interactions between pocket proteins and E2F family members are more complex than hereto anticipated and suggests cross-talk between these pathways.

RESULTS

We were interested in determining the effects that expression of G1 cyclins have on the phosphorylation status of endogenous pocket proteins and the subsequent effects on pocket protein-E2F complexes, expression of E2F-dependent and independent gene products, and, ultimately, entry and progression throughout the cell cycle. To this end, we chose an immor-
tal human cell line (T98G cells), which has been extensively used in cell cycle studies. We also used the rat embryo fibroblast cell line Rat-1. Both T98G and Rat-1 cells are effectively arrested in a quiescent state by serum starvation, and subsequent mitogenic stimulation leads to synchronous cell cycle entry. To ectopically express G1 cyclins and cyclin kinase inhibitors, we utilized replication-defective recombinant adenoviruses. Under our experimental conditions, virtually all cells are transduced by the recombinant adenoviruses at multiplicity of infection values of 50–100 as estimated by using recombinant adenoviruses encoding the green fluorescent protein and β-galactosidase (data not shown). Initial experiments demonstrated that transduction of serum-starved T98G cells with recombinant adenoviruses encoding cyclins D1 (Ad-Cyc D1) or E (Ad-Cyc E), but not β-galactosidase control adenoviruses (Ad-β-gal), for 48 h was sufficient to induce cell cycle entry in the absence of any mitogenic stimuli as determined by fluorescence-activated cell sorting analysis following propidium iodide staining (data not shown; see below). This was also confirmed using Rat-1 cells.

To determine the effects of ectopic expression of cyclin D1 and E alone or combined on cell cycle entry, T98G cells were serum-starved for 2 days and then stimulated with FBS or infected with the indicated recombinant adenoviruses at a multiplicity of infection of 50 (Fig. 1). Cells were harvested at the indicated time points and processed for fluorescence-activated cell sorting and Western blot analysis. The expression of the ectopically expressed cyclins is shown in Fig. 1A. Cells maintained in the absence of serum did not show changes in cell cycle distribution (Fig. 1B). Similar results were obtained with cells transduced with the β-galactosidase control adenovirus in the absence of serum (data not shown; see below). As expected, stimulation with 10% FBS led to synchronous cell cycle entry. More than 40% of the cells were in S phase by 22 h, and by 30 h most cells were in the next G1 phase following mitosis (Fig. 1B). Interestingly, ectopic expression of cyclin D1 and cyclin E individually or together led to synchronous cell cycle entry and progression throughout S phase. Cyclin D1 appeared more potent in inducing cell cycle progression than cyclin E (compare panels 3 and 4). Moreover, when both cyclins were coexpressed, the kinetics of cell cycle progression were very similar to that induced by cyclin D1 alone (compare panels 3 and 5). To determine whether the expression of cyclins D1 and E alone or combined was able to induce changes in the patterns of phosphorylation of pocket proteins similar to those changes induced by stimulation with FBS, we resolved protein extracts by 6% SDS-PAGE (Fig. 1C).
Fig. 2. Ecotropic coexpression of cyclins and cyclin kinase inhibitors leads to individual up-regulation of G1 CDK activities. 72 h after serum starvation, cells were treated as described under “Experimental Procedures” and as indicated in the figure. Cells were stimulated by the addition of 10% FBS to the medium or infected with the indicated combinations of adenoviruses. A, cells were harvested and lysed at 15, 22, and 30 h as indicated, and protein extracts were probed by Western blot analysis with antibodies against cyclin D1, cyclin E, p16, and p21 to verify ectopic expression of the transducted genes. B, protein lysates from samples collected at the indicated time points were pulled down with cyclin D1 (upper panel), cyclin E (middle panel) and CDK2 (lower panel) antibodies. The kinase activity associated with the immunoprecipitates was determined by using GST-pRB-CTD or histone H1 as exogenous substrates, as indicated.

SDS-PAGE followed by Western blot analysis with specific antibodies. Fig. 1C shows that expression of both cyclins appears sufficient to induce hyperphosphorylation of the three pocket proteins (see below). p130-phosphorylated forms found in quiescent T98G cells consist of forms 1 and 2 (12, 32). Stimulation with FBS triggers phosphorylation of p130 to form 3 in mid-G1 (8–10 h in T98G cells) (Fig. 1C, panel 2) (12). p107 is expressed at low levels, and it is found in its hypophosphorylated form in quiescent cells. p107 becomes hyperphosphorylated and expressed at higher levels concomitantly with p130 hyperphosphorylation. pRB also becomes hyperphosphorylated in mid-G1. (Note that the anti-pRB mixture of monoclonal antibodies used in this experiment had a preference for the hypophosphorylated form; whereas the appearance of hyperphosphorylated forms is clear, the change in intensity of the different forms does not reflect a change in the expression of pRB.) Ectopic expression of cyclin D1 in the absence of FBS was apparently sufficient to induce the same changes in protein phosphorylation induced by serum stimulation (Fig. 1C, compare panels 2 and 3). Of note, cyclin E appeared less effective in inducing changes in pocket protein phosphorylation and cell cycle progression. Finally, coexpression of cyclins D1 and E exhibited the same effects of cyclin D1 alone. The results of this experiment demonstrate that ectopic expression of either cyclin D1 or cyclin E alone is sufficient to bypass the growth factor requirements necessary to trigger hyperphosphorylation of pocket proteins and cell cycle entry in serum-starved quiescent T98G cells. However, because activation of cyclin D1-CDK complexes is likely to lead to activation of endogenous cyclin E-CDK2 complexes and vice versa, we could not discern the individual effects of each cyclin on pocket protein phosphorylation and cell cycle progression.

Similar experiments were also performed using Rat-1 cells. The expression of either cyclin D1 or cyclin E was sufficient to trigger phosphorylation of pocket proteins and cell cycle progression in the absence of serum (data not shown).

Induction of Individual G1 CDK Activities in Quiescent T98G Cells—A strategy to induce activation of cyclin D1-CDK complexes in the absence of CDK2 activity or activation of cyclin E-CDK2 complexes in the absence of D-type cyclin-CDK activity was designed. Our strategy consists of transducing serum-starved T98G cells with recombinant adenoviruses expressing either p16 (an inhibitor of D-type cyclin-CDK activity) (39), p21 (an assembly factor for D-type cyclin-CDK complexes and a potent inhibitor of cyclin E-CDK2 complexes) (reviewed in Ref. 20), or Ad-β-gal (control). 5 h after transduction, (a) the cells infected with Ad-p16 were infected with Ad-Cyc E, (b) the cells infected with Ad-p21 were infected with Ad-Cyc D1, and (c) the cells infected with Ad-β-gal were infected with Ad-Cyc D1, Ad-Cyc E, or Ad-β-gal. These combinations were predicted to generate both individual and cooperative G1 CDK activities in vivo. To determine whether this was the case, cells were harvested at the indicated time points after transduction with the second group of recombinant adenoviruses (Fig. 2). Whole cell protein lysates were obtained and used to perform kinase assays. Fig. 2A shows efficient expression of the ectopically expressed proteins, as determined by Western blot analysis. Under the shown exposure times, endogenous cyclin D1 is not detected (see Fig. 1). We determined the kinase activities associated with cyclin D1, cyclin E, and CDK2 by immunoprecipitating the kinase complexes with specific antibodies and performing kinase assays with the immunoprecipitates using the C-terminal domain of pRB and histone H1 as exogenous substrates as indicated in Fig. 2B. As expected, stimulation of T98G cells with FBS led to up-regulation of cyclin D1-, cyclin E-, and CDK2-associated kinase activities (lanes 1–3). Ectopic expression of cyclin D1 resulted in high levels of cyclin D1-associated kinase activity, which was followed by induction of cyclin E- and CDK2-associated kinase activities (lanes 10 and 11). The differences between cyclin E- and CDK2-associated kinase activities are that the latter consists of cyclin E-CDK2 and cyclin A-CDK2 activities. Of note, coexpression of cyclin D1 and p21 also leads to induction of cyclin D1-associated kinase activity to levels comparable with those induced by serum.

Cells—A strategy to induce activation of cyclin D1-CDK complexes in the absence of CDK2 activity or activation of cyclin E-CDK2 complexes in the absence of D-type cyclin-CDK activity was designed. Our strategy consists of transducing serum-starved T98G cells with recombinant adenoviruses expressing either p16 (an inhibitor of D-type cyclin-CDK activity) (39), p21 (an assembly factor for D-type cyclin-CDK complexes and a potent inhibitor of cyclin E-CDK2 complexes) (reviewed in Ref. 20), or Ad-β-gal (control). 5 h after transduction, (a) the cells infected with Ad-p16 were infected with Ad-Cyc E, (b) the cells infected with Ad-p21 were infected with Ad-Cyc D1, and (c) the cells infected with Ad-β-gal were infected with Ad-Cyc D1, Ad-Cyc E, or Ad-β-gal. These combinations were predicted to generate both individual and cooperative G1 CDK activities in vivo. To determine whether this was the case, cells were harvested at the indicated time points after transduction with the second group of recombinant adenoviruses (Fig. 2). Whole cell protein lysates were obtained and used to perform kinase assays. Fig. 2A shows efficient expression of the ectopically expressed proteins, as determined by Western blot analysis. Under the shown exposure times, endogenous cyclin D1 is not detected (see Fig. 1). We determined the kinase activities associated with cyclin D1, cyclin E, and CDK2 by immunoprecipitating the kinase complexes with specific antibodies and performing kinase assays with the immunoprecipitates using the C-terminal domain of pRB and histone H1 as exogenous substrates as indicated in Fig. 2B. As expected, stimulation of T98G cells with FBS led to up-regulation of cyclin D1-, cyclin E-, and CDK2-associated kinase activities (lanes 1–3). Ectopic expression of cyclin D1 resulted in high levels of cyclin D1-associated kinase activity, which was followed by induction of cyclin E- and CDK2-associated kinase activities (lanes 10 and 11). The differences between cyclin E- and CDK2-associated kinase activities are that the latter consists of cyclin E-CDK2 and cyclin A-CDK2 activities. Of note, coexpression of cyclin D1 and p21 also leads to induction of cyclin D1-associated kinase activity to levels comparable with those induced by serum.
stimulation (compare lanes 2 and 3 with lanes 12 and 13). However, cyclin D1 kinase activity was lower in cells coexpressing cyclin D1 and p21 than in cells expressing cyclin D1 alone (compare lanes 10 and 11 with lanes 12 and 13). This suggests that p21, when expressed at high levels, inhibits cyclin D1-CDK activity (20). Importantly, cyclin D1 activation in cells coexpressing p21 is not accompanied by activation of cyclin E or CDK2-associated kinase activities (compare lanes 10 and 11 with lanes 12 and 13). Thus, cotransfection of cyclin D1 and p21 results in individual activation of cyclin D1-CDK complexes. An independent strategy to activate D-type cyclin-CDK activity in the absence of cyclin E-CDK2 activity consisted of treating cells expressing exogenous p16 and cyclin E with roscovitine, an inhibitor of CDK2/CDC2 (see below).

On the other hand, expression of cyclin E leads to induction of cyclin E and CDK2-associated kinase activities and, to a lesser extent, cyclin D1-associated kinase activity (compare lanes 6 and 7 with lanes 4 and 5). Notably, coexpression of cyclin E and p16 prevented activation of cyclin D1 kinase activity but had little effect on the cyclin E- and CDK2-associated kinase activities (compare lanes 6 and 7 with lanes 8 and 9). Thus, we can selectively induce activation of cyclin E-CDK2 complexes without inducing D-type cyclin-CDK complexes.

**Effects of Collective or Selective Activation of G1 Cyclin-CDK Activities on the Phosphorylation Status of Endogenous Pocket Proteins in the Absence of Mitogens**—Next, we determined the effects that collective or selective activation of G1 cyclin-CDK activities had on the phosphorylation status of the three endogenous pocket proteins in comparison with the changes induced by stimulation with FBS at the indicated time points. Fig. 3A shows that whereas expression of cyclin D1 leads to hyperphosphorylation of the three pocket proteins, coexpression of cyclin D1 with p21, which blocks CDK2 activity, prevents hyperphosphorylation of both p130 and pRB (compare lanes 14–16 with lanes 17–19). Interestingly, under these same conditions, p107 is efficiently hyperphosphorylated. However, the induction of p107 protein expression, which is believed to result from disruption of E2F-dependent repression of the p107 gene promoter, is mostly inhibited (see below). Similar results were obtained when T98G cells transduced with cyclin D1 were treated with roscovitine, which inhibits CDK2/CDC2 activity (Fig. 3B). Roscovitine blocked p107 up-regulation and inhibited S phase entry, but cyclin D1-induced hyperphosphorylation of p107 was undisturbed. Thus, the same result was obtained using two different strategies that block cyclin E-CDK2 activity in cyclin D1-overexpressing cells.

Similarly, as shown in Fig. 1C, cyclin E expression is sufficient to induce hyperphosphorylation of the three pocket proteins (Fig. 3A, lanes 8–10), although it appears significantly less potent than cyclin D1 (Fig. 3A, compare lanes 8–10 with

---

**Fig. 3. Complete hyperphosphorylation of pRb and p130, but not p107, requires cooperation of cyclin D1- and cyclin E-associated kinase activities.** However, cyclin D1-CDK activity is sufficient to hyperphosphorylate p107. Cell lysates were obtained as in Fig. 2 and resolved in 6% SDS-PAGE to clearly separate the different phosphorylated forms of each pocket protein. A, immunodetection of p130 (upper), p107 (middle) and pRb (lower panel). p130 phosphorylated forms are indicated as described earlier (12, 32) and subsequent studies. p107-P and pRb-P, hyperphosphorylated p107 and pRB, respectively. *, phosphorylated form 2b of p130 (12, 40), present in cells expressing exogenous p16 and cyclin E. B, T98G cells transduced with cyclin D1 adenoviruses for the indicated periods of time were incubated in the presence of the CDK2 inhibitor roscovitine or vehicle (Me2SO; DMSO). Protein lysates were analyzed by Western blot (WB) by using anti-p107 antibodies. The upper and lower panels show two different exposures from the same Western blot to allow visualization of hyper- and hypophosphorylated forms of p107, which are expressed at very different levels (L denotes long exposure and S denotes short exposure). The percentage of cells in S phase was determined by flow cytometric analysis following propidium iodide staining.
of cyclin E and p16, which results in activation of cyclin E-CDK2 complexes in the absence of D-type cyclin-CDK activity, leads only to partial phosphorylation of p130 and pRB. Under the same conditions, p107 appears to remain in its hypophosphorylated form, but induction of its protein expression is unperturbed (Fig. 3A, lanes 11–13). p130 is partially hyperphosphorylated to form 2b (indicated by an asterisk), a partially phosphorylated form of p130 that we initially identified in 293 cells (12) and later found in other E1A-expressing cells (40). p130 form 2b migrates faster than form 3, but slightly more slowly than form 2. It is now established that this form of p130 is detected in cells in which p-type cyclin activity is inhibited by different mechanisms (41, 42). These results also highlight that even when G1 cyclins are overexpressed, the resulting CDK activities continue to exhibit selectivity in the phosphorylation of pocket proteins.

The differential phosphorylation of the three pocket proteins by the selective activation of G1 CDK activities suggests that the disruption of pocket protein-E2F interactions also might be differentially affected by these kinase activities.

**Regulation of Pocket Protein/E2F Interactions by G1 CDKs**—To explore this possibility, we first determined changes in p130/E2F4 interactions by performing immunoprecipitations followed by Western blot analysis using specific antibodies. Fig. 4A shows that 15 h following serum stimulation, p130/E2F4 complexes are mostly disrupted as a result of p130 hyperphosphorylation to form 3, as previously described (32). Expression of cyclin D1 in the absence of serum was sufficient to completely disrupt these complexes (Fig. 4A, lanes 10 and 11). However, coexpression of p21 effectively blocked complex disruption, strongly indicating that cyclin D1- and cyclin E-associated kinase activities cooperate to abrogate this interaction (Fig. 4A, compare lanes 10 and 11 with lanes 12 and 13). Expression of cyclin E alone led to partial disruption of p130/E2F4 complexes, although it was less efficient than cyclin D1 (Fig. 4A, compare lane 1 with lanes 6 and 7 and lanes 10 and 11). p16 had little effect on cyclin E-mediated disruption of these complexes (Fig. 4A, lanes 8 and 9). Altogether, these results demonstrate that cyclin D1-CDK and cyclin E-CDK2 complexes cooperate to disrupt p130/E2F4 complexes. It is also shown that cyclin E-CDK2 without cyclin D1-CDK activity, but not cyclin D1-CDK without cyclin E-CDK2 activity, can induce at least partial disruption of p130-E2F4 complexes.

We next determined the regulation of p107-E2F4 and pRB-E2F4 complexes in comparison with p130-E2F4 complexes (Fig. 4B, upper panels). Interestingly, conditions leading to abrogation of p130-E2F4 complexes led to similar disruption of pRB-E2F4 complexes. In contrast, p107-E2F4 complexes accumulated following disruption of p130-E2F4 and pRB-E2F4 complexes as a result of serum stimulation or activation of G1 CDK activities. This indicates that although hyperphosphorylation of p130 and pRB is very effective at disrupting their interaction with E2F4, the increased expression of p107 appears to facilitate that a pool of hypophosphorylated p107 remains available for interaction with E2F4.

We then analyzed the formation/abrogation of pocket protein-E2F1 complexes (Fig. 4B, lower panels). Four major observations were made. First, disruption of pRB-E2F4 complexes coincides with formation of pRB-E2F1 complexes. Second, at least some partially hyperphosphorylated forms of pRB are detected in association with E2F1, although there is a selective preference for the hypophosphorylated form (compare pRB forms in E2F1 immunoprecipitates and E2F1-depleted lysates) (Fig. 4B, lower panels, and Fig. 4C; see also Fig. 5B). Third, and more surprisingly, disruption of p130-E2F4 and pRB-E2F4 complexes is followed by formation of p130-E2F1 and p107-E2F1 complexes (Fig. 4B). Fourth, the primary form of p130 that interacts with E2F1 is hyperphosphorylated and co-migrates with form 3 (longer exposures revealed trace levels of forms 1 and 2 migrating faster than the major form associated with E2F1) (data not shown). In addition, the migration of the p130 form associated with E2F1 matches precisely the migration of p130 form 3 detected in E2F1 immunodepleted lysates (Fig. 4, B and C). Of note, the kinetics of association of pocket proteins with E2F1 is distinct as can be seen by comparing the patterns of pocket protein/E2F1 association induced by serum and transduction with cyclin D1 adenoviruses (Fig. 4B). Notably, we did not observe formation of p130-E2F1 and p107-E2F1 complexes in cells transduced with cyclin E, which is consistent with the diminished ability of cyclin E in inducing abrogation of p130-E2F4 and pRB-E2F4 complexes and the lesser up-regulation of E2F1 (Figs. 4, A and B, and 6A).

To confirm the specificity of the E2F1 interaction with p130 and p107, we performed additional immunoprecipitations with various different antibodies. Fig. 5A shows that p107 is specifically coimmunoprecipitated with E2F1, since the antigenic peptide used to raise the C20 anti-E2F1 polyclonal antibody blocks immunoprecipitation of p107-E2F1 complexes. The C20 polyclonal antibody was raised against a 20-amino acid peptide corresponding to the C terminus of E2F1. We also performed immunoprecipitations using a monoclonal antibody specific for E2F1, KH95, which has been extensively used for the analysis of E2F1 complexes. This antibody confirmed the results obtained with the C20 anti-E2F1 polyclonal antibody. KH95 effectively immunoprecipitated p107 and pRB with E2F1. p130 was detected less efficiently (Fig. 5B). Moreover, anti-p107 antibodies also coimmunoprecipitated E2F1, which was detected by Western blot analysis with both C20 polyclonal and KH95 monoclonal anti-E2F1 antibodies (Fig. 5C). Similarly, anti-p130 antibodies also coimmunoprecipitated E2F1 (data not shown). Thus, an unexpected interaction between p130/p107 and E2F1 was observed in vivo, which results from changes in G1 CDK-induced cell cycle-dependent expression of p107 and E2F1 as well as disruption of p130-E2F4 complexes.

Previous studies have failed to detect interactions between endogenous p107/p130 and E2F1 (reviewed in Ref. 14). This is likely because these complexes are not very abundant and are only transiently formed at specific times during the cell cycle. It is also possible that E2F1 has a higher affinity for pRB than for p107/p130. Our strategy to detect these complexes was based on (a) using cells synchronized at specific points of the cell cycle to prepare the protein extracts; (b) preparing protein extracts in DIP buffer, which was previously described for the efficient immunoprecipitation of active D-type cyclin-CDK complexes (36); and (c) performing immunoprecipitations with anti-E2F1 antibodies by using up to 500 μg of protein extracts.

**Changes in the Expression of E2F-dependent and -independent Genes—**Given that activation of G1 cyclin-CDK activities exhibit specific effects on the regulation of pocket protein-E2F2 complexes, we analyzed changes in E2F-dependent gene expression. The expression of p107, E2F1, cyclin E, and cyclin A was determined by Western blot analysis, since it is known that the cell cycle-dependent up-regulation of these four proteins is regulated at the transcriptional level by E2F activities (Fig. 6A). p107 Western blots are shown again for clarity and to allow for direct comparison of changes in expression. (The same p107 Western blots were included in Fig. 3A to show changes in phosphorylation.) Serum stimulation clearly leads to the up-regulation of the four proteins, although with different kinetics (Fig. 6A, panel 1). Ectopic expression of cyclin D1 induces similar up-regulation of the four proteins, although there are noticeably slower kinetics in comparison with FBS.
stimulation, which is clearest in the case of cyclin A up-regulation (Fig. 6A, panel 5). This is consistent with slower progression through the cell cycle (see Fig. 1 and below). Interestingly, coexpression of p21 effectively blocked the up-regulation of all four proteins (Fig. 6A, panel 6), which is in agreement with the inability of cyclin D1 to disrupt E2F4-containing complexes in

**G1 cyclin-CDK-dependent modulation of pocket protein/E2F interactions.** p130 and p107 form specific complexes with E2F1. A, protein extracts obtained as in Fig. 2 were immunoprecipitated using specific antibodies against E2F4 (upper panel) and p130 (middle panel), and immunocomplexes were resolved in 8% SDS-PAGE, blotted, and probed with p130 (upper panel) and E2F4 (middle and lower panels) antibodies. The upper and middle panels show p130-E2F4 complexes. The lower panel shows E2F4 protein levels in total cell lysates. B, protein extracts from the indicated time points were immunoprecipitated with anti-E2F4 (upper panels) or anti-E2F1 (lower panels), and immunocomplexes were resolved in 8% SDS-PAGE, blotted, and probed with the indicated antibodies. pRB-pP, partially phosphorylated pRB. The migration of the IgGs is also indicated for the E2F4 Western blot. C, protein lysates that had been immunodepleted with anti-E2F1 antibodies were resolved in 8% SDS-PAGE in parallel with the immunocomplexes shown in B, blotted, and probed with anti-p130 and anti-pRB antibodies. Differently phosphorylated forms of pocket proteins are indicated as in Fig. 1C.

**FIG. 4.** G1 cyclin-CDK-dependent modulation of pocket protein/E2F interactions. p130 and p107 form specific complexes with E2F1. A, protein extracts obtained as in Fig. 2 were immunoprecipitated using specific antibodies against E2F4 (upper panel) and p130 (middle panel), and immunocomplexes were resolved in 8% SDS-PAGE, blotted, and probed with p130 (upper panel) and E2F4 (middle and lower panels) antibodies. The upper and middle panels show p130-E2F4 complexes. The lower panel shows E2F4 protein levels in total cell lysates. B, protein extracts from the indicated time points were immunoprecipitated with anti-E2F4 (upper panels) or anti-E2F1 (lower panels), and immunocomplexes were resolved in 8% SDS-PAGE, blotted, and probed with the indicated antibodies. pRB-pP, partially phosphorylated pRB. The migration of the IgGs is also indicated for the E2F4 Western blot. C, protein lysates that had been immunodepleted with anti-E2F1 antibodies were resolved in 8% SDS-PAGE in parallel with the immunocomplexes shown in B, blotted, and probed with anti-p130 and anti-pRB antibodies. Differently phosphorylated forms of pocket proteins are indicated as in Fig. 1C.

**G1 CDKs in Cell Cycle-regulated E2F-dependent Gene Expression**
the absence of cyclin E-CDK2 activity (compare Figs. 2, 3, 4, and 6A). Ectopic expression of cyclin E also led to the expression of p107, E2F1, and cyclin A (Fig. 6A, panel 3). Importantly, coexpression of cyclin E with p16, which effectively blocks α-type cyclin-associated kinase activity, did not effectively block the up-regulation of p107, E2F1, and cyclin A induced by cyclin E expression (Fig. 6A, panel 4). Thus, activation of cyclin E-CDK2 activity in the absence of α-type cyclin-CDK activity is sufficient to induce expression of E2F-dependent genes. Notably, accumulation of hypophosphorylated p107 does not prevent the expression of E2F-dependent genes (Fig. 6A, panel 4) or cell cycle progression, although it might delay these processes.

Whereas it is clear that G1 cyclin-CDK complexes are involved in regulating the expression of E2F-dependent genes, these CDKs also regulate the expression of other cell cycle-regulatory proteins. Fig. 6B shows the expression of p27. It is well known that serum stimulation leads to p27 down-regulation, which facilitates the activation of cyclin E-CDK2 complexes. Interestingly, ectopic expression of cyclin D1 was sufficient to induce partial down-regulation of p27 (Fig. 6B, compare lane 1 with lanes 10 and 11). Interestingly, cyclin D1-mediated down-regulation of p27 did not require the activity of CDK2 complexes, since p21 expression did not effectively block p27 down-regulation (Fig. 6B, lanes 12 and 13). Cyclin E expression, however, failed to consistently induce down-regulation of p27 (Fig. 6B, lanes 6–9).

**Mitogenic Independent Growth**—Finally, we determined the cell cycle and apoptotic effects associated with the cooperative
or selective activation of G1 cyclin-CDK complexes. Cell cycle progression was determined by flow cytometric analysis following propidium iodide staining (Fig. 7A). Ectopic expression of cyclin D1 induced growth factor-independent cell cycle progression through G1, S phase, and mitosis as well as entering the next G1 phase of the cell cycle. Entry into the following G1 was detectable by 30 h. This was entirely inhibited by coexpression of p21. Similarly, we showed that roscovitine inhibited cyclin D1-induced entry into S phase (Fig. 3B). Cyclin E expression led to progression through G1 and S and entry into mitosis. Coexpression of p16 did not block cyclin E-mediated induction of cell cycle progression.

We also determined whether activation of G1 cyclin-CDK activities was sufficient to confer growth factor-independent cell cycle division through multiple generations. To this end, we used two assays: (a) a nocodazole arrest, to assess accumulation of cells in mitosis, and (b) an assay that is readily used to count cell divisions of lymphocytes (CFSE content assay). In the first assay, T98G cells were serum-stimulated or transduced with the indicated adenoviruses as in Fig. 7A. 22 h later, nocodazole was added to the medium, and cells were incubated for 22 h. Cells were collected and prepared for flow cytometric analysis. It is clear that both cyclin D1 and cyclin E can induce accumulation of cells with a 4n DNA content (Fig. 7B). This indicates that these cells completed DNA replication successfully. Obviously, cells transduced with cyclin D1 and p21 remained in G1. This experiment also shows clearly that most cells stimulated with FBS or transduced with cyclin D1 were in mitosis. In contrast, a significant proportion of the cells transduced with cyclin E were still progressing through the G1 and S phases. Nocodazole release resulted in entry of the cells into the next G1 phase, although apoptosis was observed (Fig. 7B, lower right panel, and see below). In the second assay, serum-starved T98G cells were incubated with CFSE. Next, we either stimulated cells with FBS or infected them as in the previous set of experiments. At the indicated time points, cells were collected, and the CFSE content per cell was determined by flow cytometry. Quiescent (non-treated) cells were considered the parental population. At every division, CFSE segregates into daughter cells so that each generation shows less fluorescence per cell. The percentage of cells corresponding to each generation is shown. Black bars, parental population; light gray bars, second generation; white bars, third generation. D, apoptosis was assessed by detecting PARP cleavage at 2 and 3 days post-treatments. Protein extracts corresponding to the indicated time points were resolved by SDS-PAGE followed by Western blot analysis using specific anti-PARP antibodies. The migration of the 85-kDa cleaved product is indicated.

![Fig. 7](image-url)
cells transduced with the various combinations of adenoviruses were cycling more slowly than cells stimulated with FBS. Although it is not obvious from direct observation, there is also the possibility that a small portion of cells were dying by apoptosis due to the absence of survival factors present in serum. Thus, we determined induction of apoptosis by analyzing PARP cleavage. Fig. 7D shows that ectopic expression of G1 cyclin in the absence of serum results in perceptible apoptosis as detected by PARP cleavage (appearance of an 87-kDa cleaved PARP fragment). PARP cleavage was more evident in cells ectopically expressing cyclin D1. In agreement with this result, we also observed apoptosis in cyclin D1-transduced T98G cells entering G1 upon release from a nocodazole arrest (data not shown).

**DISCUSSION**

In this report, we show that expression of both cyclin D1 and cyclin E are sufficient to induce growth factor-independent cell cycle progression in human T98G and Rat-1 cells. Whereas cyclin E expression can induce cell cycle progression in the absence of d-type-CDK activity, activation of cyclin D1-CDK complexes in the absence of cyclin E-CDK2 activity is not sufficient to induce DNA synthesis. However, we have observed that expression of cyclin D1 alone was as potent as expression of both cyclin D1 and E in inducing phosphorylation of pocket proteins and changes in E2F-dependent gene expression and cell cycle progression (Table I).

Ectopic expression of cyclin D1 leads to downstream activation of endogenous cyclin E-CDK2 complexes and induces cell cycle progression more potently than cyclin E. In agreement with the more potent effects of ectopically expressing cyclin D1, the overall CDK2 activity, but not the cyclin E-CDK2 activity, is higher than in those cells ectopically expressing cyclin E, presumably because cyclin D1 induces activation of cyclin A-CDK2 complexes more effectively than cyclin E. This is likely to result from a combination of events induced by cyclin D1, which includes more effective up-regulation of the cyclin A protein, increased down-regulation of p27, and increased sequestration of p27. In contrast, whereas ectopic expression of cyclin E induces its associated kinase activity to levels similar to or higher than serum stimulation, the total CDK2 activity in cyclin E-transduced cells is lower than in FBS-treated cells. This is presumably because cyclin E induces downstream effects, such as activation of cyclin A-CDK2 complexes, less effectively than cyclin D1. Altogether, this suggests that although cyclin D1-mediated activation of endogenous cyclin E-CDK2 complexes is essential for cell cycle progression, cyclin D1 plays other roles that contribute to timely progression through G1/S phases.

Our data show that whereas transduction of either cyclin D1 or cyclin E is sufficient to induce coordinated hyperphosphorylation of the three endogenous pocket proteins, individual activation of cyclins D1 and E leads to distinct phosphorylation patterns. Both cyclins D1 and E are required for full hyperphosphorylation of pRB and p130. In contrast, cyclin D1 individually, but not cyclin E, can induce hyperphosphorylation of p107. Phosphorylation of p130 to form 2b by cyclin E-CDK2 complexes in the absence of cyclin E-CDK2 activity is not sufficient to induce DNA synthesis. However, we have observed that expression of cyclin D1 alone was as potent as expression of both cyclin D1 and E in inducing phosphorylation of pocket proteins and changes in E2F-dependent gene expression and cell cycle progression (Table I).
complexes is sufficient to disrupt p130-E2F4 complexes. This is accompanied by the expression of a number of genes whose promoters are thought to be regulated by p130 and pRB including E2F1, cyclin A, and p107 (29, 43). Of note, changes in the phosphorylation status of p107 can be uncoupled from changes in its protein expression levels. Cyclin D1 individually induces hyperphosphorylation of p107 without affecting its protein expression, whereas cyclin E induces p107 up-regulation without inducing p107 hyperphosphorylation. Importantly, cyclin E-induced dramatic accumulation of hypophosphorylated p107 does not prevent E2F-dependent gene expression or cell cycle progression, although we cannot rule out the possibility that the accumulation of hypophosphorylated p107 slows down cell cycle progression.

Our data suggest that induction of cell cycle progression by cyclin E in the absence of α-type cyclin CDK activity is coupled to the expression of a number of E2F-dependent genes. Previous studies have shown that cyclin E can override a G1 growth arrest induced by a phosphorylation-deficient pRB mutant that lacks certain CDK phosphorylation sites (22, 23). This suggested that cyclin E was capable of inducing cell cycle progression independently of the pRB/E2F2 pathway. Our data suggest that cyclin E is sufficient to abrogate the ability of both p130 and pRB to inhibit E2F4. Recent reports indicate that the p130/p107 and pRB pathways are two separate pathways required for G1 control (44–46). Thus, it would be important to examine whether ectopic expression of cyclin E in cells expressing a phosphorylation-deficient pRB mutant is sufficient to induce the expression of at least certain E2F-dependent genes such as cyclin A and/or E2F1. A potential scenario is that cyclin E overrides a G1 growth arrest induced by a phosphorylation-deficient pRB mutant because it bypasses the need for E2F activities that are specifically controlled by pRB and negatively regulate the expression of the cyclin E gene. Ectopic expression of cyclin E under these conditions is likely to be sufficient to disrupt p130-E2F4 complexes and allow for the expression of genes such as cyclin A and/or E2F1.

Unexpectedly, we found that endogenous E2F1 interacts with both p130 and p107. This interaction is modulated during the cell cycle in a manner that appears to depend on previous abrogation of p130-E2F4 and pRB-E2F4 complexes, which releases free p130 and up-regulates p107 and E2F1. In this regard, it is important to point out that E2F1 appears to interact with p130 form 3, a form of p130 that does not interact with E2F4. Thus, the availability of free p130 and the increased expression of E2F1 results in the formation of a transient complex, which probably disappears as result of p130 down-regulation. Altogether, this suggests that the interactions between pocket proteins and E2F family members are more complex and interdependent than initially anticipated. Whereas the overall levels of p130-E2F1 and p107-E2F1 complexes in the cell might be low in comparison with pRB-E2F1 complexes, it is conceivable that the former complexes play a critical role in the regulation of certain E2F1 target genes. These results are not in disagreement with previous chromatin immunoprecipitation analysis of promoter occupancy in vivo in these and other cells (43, 47). E2F4 and p130 were the major E2F and pocket proteins bound to the promoters of E2F1, p107, and cyclin A in G0 and early G1. These promoters are bound by E2F1-3, but not p130 or E2F4, in late G1 and S phases. Thus, it is conceivable that the E2F1-p130 and E2F1-p107 complexes that we have detected do not bind to these promoters. Instead, p130 and p107 may prevent binding of a portion of E2F1 to responsive promoters during late G1 and perhaps S phase. Similarly, pRB has not been detected bound to any natural E2F promoters in vivo.

One question that remains is why cells of different origins exhibit distinct sensitivity to overexpression of G1 cyclins. For instance, it has been reported that ectopic expression of G1 cyclins in rodent and human fibroblasts accelerates the G1 phase of the cell cycle but does not result in mitogen-independent growth (15–17, 48). In contrast, expression of cyclin D1 in primary rat hepatocytes or human MCF-7 breast cancer cells is sufficient to induce mitogen-independent growth or growth in medium containing very limited serum, respectively (33, 49). Thus, the most likely scenario is that the effects of overexpression of G1 cyclins in different cells depend on the efficient activation of their associated CDKs. In many cells, activation of G1 cyclin-CDK activities depends on steps that cannot be overridden simply by overexpression of their regulatory subunits. Of note, our results using Rat-1 cells are different from a previous study (16). In this previous report, overexpression of either cyclin D1 or E did not result in mitogen-independent growth. It is likely that the difference resides in the level of expression of the exogenous cyclins, which is presumably higher in our study.

Finally, our data also suggest that if constitutive activation of either cyclin D1-CDK or cyclin E-CDK2 complexes is achieved in quiescent cells, these are not only able to synthesize DNA as reported earlier (21) but are also able to grow independently of mitogens for multiple generations. This has in vivo implications for cells exhibiting certain genetic properties, because hyperactivation of G1 cyclins as a result of mutations might result in autonomous growth in the absence of growth factors.

Acknowledgments—We thank Jeffry Albrecht, Juan Pueyo, Pilar Ruiz-Lozano, and Wafik El-Deiry for providing recombinant adenoviruses and Ed Harlow for antibodies. We thank Dale Haines, Peter Adams, Renee Marshall, Sahyansachi Bhattacharya, and Dominic Salerno for critically reading the manuscript and Rosemary Dillon for manuscript editing. We thank Mayumi Kataoka for Flow cytometric analysis and May Truongcao and Julita Yarwood for technical assistance.

REFERENCES

1. Peeper, D. S., Upton, T. M., Ladhwa, M. H., Neuman, E., Zalveske, J., Bernards, R., DeCaprio, J. A., and Ewen, M. E. (1997) Nature 386, 177–181
2. Mittnacht, S., Paterson, H., Olson, M. F., and Marshall, C. J. (1997) Curr. Biol. 7, 219–221
3. Wignall, J. T., Coats, S. R., Wang, Y. Z., and Pledger, W. J. (1996) Oncogene 12, 127–134
4. Liu, J. F., Chao, J. R., Jiang, M. C., Ng, S. Y., Yen, J. Y., and Yang-Yen, H. F. (1995) Mol. Cell. Biol. 15, 3654–3663
5. Leone, G., DeGregori, J., Sears, R., Jakoi, L., and Nevin, J. R. (1997) Nature 387, 422–426
6. Buchkovich, K., Duffy, L. A., and Harlow, E. (1988) Cell 58, 1097–1105
7. Chen, P. L., Scully, P., Shew, J. Y., Wang, J. Y., and Lee, W. H. (1988) Cell 58, 1193–1198
8. DeCaprio, J. A., Ladlow, J. W., Lynch, D., Furukawa, Y., Griffin, J., Pimwornus, H., Huang, C. M., and Livingston, D. M. (1989) Cell 58, 1085–1095
9. Mihara, K., Cao, X. R., Yen, A., Chandler, S., Driscoll, B., Murphy, A. L., T’Ang, A., and Fung, Y. K. (1989) Science 246, 1300–1303
10. Beijersbergen, R. L., Carlee, L., Kerkhoven, R. M., and Bernards, R. (1995) Genes Dev. 9, 1340–1353
11. Xiao, Z. X., Ginsberg, D., Ewen, M., and Livingston, D. M. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 4627–4632
12. Mayol, X., Garriga, J., and Grau, X. (1995) Oncogene 11, 801–808
13. Hinds, P. W., Mittnacht, S., Dulic, V., Arnold, A., Reed, S. I., and Weinberg, R. A. (1992) Cell 70, 993–1006
14. Graña, X., Garriga, J., and Mayol, X. (1998) Oncogene 17, 3365–3383
15. Quelle, D. E., Ashmun, R. A., Shurtleff, S. A., Kato, J. Y., Bar, S. D., Roussel, M. F., and Sherr, C. J. (1993) Genes Dev. 7, 1559–1571
16. Resnitzky, D., Gossen, M., Bujard, H., and Reed, S. I. (1994) Mol. Cell. Biol. 14, 1669–1679
17. Ohta, M., and Roberts, J. M. (1994) Science 260, 1908–1912
18. Resnitzky, D., and Reddy, E. B. (1995) Oncogene 11, 211–219
19. Morgan, D. O. (1995) Nature 374, 131–134
20. Sherr, C. J., and Roberts, J. M. (1999) Genes Dev. 13, 1501–1512
21. Connell-Crowley, L., Elledge, S. J., and Harper, J. W. (1997) Curr. Biol. 7, 65–68
22. Lukas, J., Herzinger, T., Hansen, K., Moroni, M. C., Resnitzky, D., Helin, K., Reed, S. I., and Barret, J. (1997) Genes Dev. 11, 1479–1492
23. Leng, X., Connell-Crowley, L., Goodrich, D., and Harper, J. W. (1997) Curr. Biol. 7, 709–712
24. Lukas, J., Parry, D., Aagaard, L., Mann, D. J., Bartkova, J., Strauss, M.,
50274  

G_1 CDKs in Cell Cycle-regulated E2F-dependent Gene Expression

Peters, G., and Bartek, J. (1995) Nature 375, 503–506
25. Koh, J., Enders, G. H., Dynlacht, B. D., and Harlow, E. (1995) Nature 375, 506–510
26. Medema, R. H., Herrera, R. E., Lam, F., and Weinberg, R. A. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 6289–6293
27. Hansen, K., Farkas, T., Lukas, J., Holm, K., Ronnstrand, L., and Bartek, J. (2001) EMBO J. 20, 422–432
28. Leng, X., Noble, M., Adams, P. D., Qin, J., and Harper, J. W. (2002) Mol. Cell. Biol. 22, 2242–2254
29. Hurford, R. K., Cobrinik, D., Lee, M. H., and Dyson, N. (1997) Genes Dev. 11, 1447–1463
30. Medema, R. H., Herrera, R. E., Lam, F., and Weinberg, R. A. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 6289–6293
31. Meijer, L., Borgne, A., Mulner, O., Chong, J. P. J., Blow, J. J., Inagaki, N., Inagaki, M., Delcros, J. G., and Moulinoux, J. P. (1997) Eur. J. Biochemistry 243, 527–536
32. Mayol, X., Garriga, J., and Graña, X. (1998) Oncogene 13, 237–246
33. Albrecht, J. H., and Hansen, L. K. (1999) Cell Growth Differ. 10, 397–404
34. Becker, T. C., Noel, R. J., Coats, W. S., Gomez-Fox, A. M., Alam, T., Gerard, R. D., and Newgard, C. B. (1994) Methods Cell Biol. 43, 161–189
35. Garriga, J., Limon, A., Mayol, X., Rane, S. G., Albrecht, J. H., Reddy, E. P., Andreu, V., and Graña, X. (1998) Biochem. J. 333, 645–654
36. Reed, M. F., Liu, V. F., Ladha, M. H., Ando, K., Griffin, J. D., Weaver, D. T., and Even, M. E. (1998) Oncogene 17, 2961–2971
37. Graña, X., De Luca, A., Sang, N., Pu, Y., Claudio, P. P., Rosenblatt, J., Morgan, D. O., and Giordano, A. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 3834–3838
38. Garriga, J., Segura, E., Mayol, X., Grubmeyer, C., and Graña, X. (1996) Biochem. J. 320, 983–989
39. Serrano, M., Hannon, G. J., and Beach, D. (1993) Nature 366, 704–707
40. Parreño, M., Garriga, J., Limon, A., Mayol, X., Beck, G. R., Jr., Moran, E., and Graña, X. (2000) J. Virol. 74, 3166–3176
41. Cheng, L., Ross, F., Fang, W., Mon, T., and Cobrinik, D. (2000) J. Biol. Chem. 275, 30317–30325
42. Parreño, M., Garriga, J., Limon, A., Albrecht, J., and Graña, X. (2001) Oncogene 1975–4866
43. Takahashi, Y., Raymon, J. B., and Dynlacht, B. D. (2000) Genes Dev. 14, 894–896
44. Bruce, J. L., Hurford, R. K., Jr., Classon, M., Koh, J., and Dyson, N. (2000) Mol. Cell 6, 737–742
45. Sage, J., Mulligan, G. J., Attardi, L. D., Miller, A., Chen, S., Williams, B., Theodoru, E., and Jacks, T. (2000) Genes Dev. 14, 3037–3050
46. Dannenberg, J. H., van Rossum, A., Schuifff, L., and te Riele, H. (2000) Genes Dev. 14, 3051–3064
47. Rayman, J. B., Takahashi, Y., Indjeian, V. B., Dannenberg, J. H., Catchpole, S., Watson, R. J., te Riele, H., and Dynlacht, B. D. (2002) Genes Dev. 16, 533–547
48. Resnitzky, D., and Reed, S. I. (1995) Mol. Cell. Biol. 15, 3463–3469
49. Zwijsen, R. M., Klompmaker, R., Wientjens, E. B., Kruijt, P. M., van der Burg, B., and Michalides, R. J. (1996) Mol. Cell. Biol. 16, 2554–2560
G₁ Cyclin/Cyclin-dependent Kinase-coordinated Phosphorylation of Endogenous Pocket Proteins Differentially Regulates Their Interactions with E2F4 and E2F1 and Gene Expression

Joaquim Calbó, Matilde Parreño, Elena Sotillo, Thomas Yong, Adela Mazo, Judit Garriga and Xavier Graña

J. Biol. Chem. 2002, 277:50263-50274. doi: 10.1074/jbc.M209181200 originally published online October 24, 2002

Access the most updated version of this article at doi: 10.1074/jbc.M209181200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 48 references, 25 of which can be accessed free at http://www.jbc.org/content/277/52/50263.full.html#ref-list-1