SV40 large T antigen (Ag) binds to all members of the retinoblastoma (RB) tumor suppressor family including pRb, p107, and p130. Although the LXCXE motif of TAg binds directly to the RB proteins, it is not sufficient to fully inactivate their function. The N-terminal DNA J domain of TAg cooperates with the LXCXE motif to override RB-mediated repression of E2F-dependent transcription. In addition, TAg can reduce the overall phosphorylation state of p107 and p130 that is dependent on an intact J domain and LXCXE motif. However, the mechanism of this activity has not been described. Here we describe the use of a cell-free system to characterize the effect of TAg on p130 phosphorylation. When incubated in extracts prepared from S phase cells, p130 undergoes specific phosphorylation. Addition of TAg to S phase extracts leads to a reduction of p130 phosphorylation in vitro. The ability of TAg to reduce the phosphorylation of p130 in vitro is dependent on an intact DNA J domain and can be inhibited by okadaic acid and PP2A-specific inhibitors. These results suggest that TAg recruits a phosphatase activity in a DNA J domain-dependent manner to reduce the phosphorylation of p130.

The RB\(^1\) family that includes pRb, p107, and p130 can inhibit cell growth, at least in part, by repressing E2F-mediated transcription. RB-mediated growth suppression is modulated by cell cycle-dependent phosphorylation and expression. For example, in G\(_0\) and early G\(_1\) phase of the cell cycle, pRb and p107 are under- or hypophosphorylated (1, 2). When cells are stimulated to enter the cell cycle, pRb and p107 become phosphorylated by the cyclin-dependent kinases (Cdk) during late G\(_1\) and S phase by cyclin D/Cdk4, cyclin E/Cdk2, and cyclin A/Cdk2 (3–6). Cell cycle-dependent phosphorylation of the RB family reduces the interaction with E2F transcription factors and permits E2F-mediated transcription during proliferation.

The pRb-related protein p130 also becomes hyperphosphorylated during the G\(_1\) to S phase transition. However, unlike pRb and p107, p130 is also phosphorylated during G\(_0\) and the early G\(_1\) phase of the cell cycle (7–9). When examined by Western blotting, p130 exists as a hypophosphorylated doublet (forms 1 and 2) in G\(_0\) and early G\(_1\) cells and as a slower migrating, hyperphosphorylated (form 3) species in late G\(_1\) and S phase cells (7–12). In addition to changes in phosphorylation, p130 levels fluctuate significantly throughout the cell cycle. Expression of p130 is relatively high in growth-arrested cells and low in rapidly proliferating cells. The decreased levels of p130 during late S phase and G\(_2\) reflect an increased rate of p130 degradation mediated by the SCFFinbp2 ubiquitin ligase (13). In contrast, pRb and p107 levels increase slightly during proliferation secondary to increased levels of mRNA expression (14, 15).

SV40 large TAg can bind and inactivate all three members of the RB family. The LXCXE motif (where X is any residue) of TAg binds directly to RB (16, 17). Mutations in the LXCXE motif that perturb TAg binding to RB reduce the transforming activity of TAg (16). Although the LXCXE motif is necessary for TAg binding to RB, it is not sufficient for complete inactivation of the RB family growth-suppressing functions. The N-terminal DNA J domain of TAg also contributes to the inactivation of the RB family. The J domain is a highly conserved motif found in cellular and viral DNA J proteins that binds and activates specific Hsp70/DnaK proteins. The N terminus of all Polyoma T antigens including the large and small TAg of SV40 contain a DNA J domain (18, 19). The J domain of SV40 TAg binds to the constitutively expressed Hsc70, contributes to viral DNA replication, and cooperates with the LXCXE motif to disrupt RB-E2F complexes (20–26). Mutation of the conserved HDP motif within the large TAg J domain disrupts binding to Hsc70 and inactivates its ability to fully inactivate p130 and p107 (22, 24).

The J domain and LXCXE motif of TAg are also required to perturb the phosphorylation state of p130 (24, 27). For example, when TAg is stably expressed in mouse embryo fibroblasts, p130 is found exclusively as a hypophosphorylated species during all phases of the cell cycle. In contrast, p130 undergoes normal cell cycle-dependent phosphorylation in cells expressing TAg that contains mutations within either the J domain or the LXCXE motif. Notably, TAg can also reduce the phosphorylation of p107 but has no apparent effect on pRb phosphorylation (24, 27). Despite these observations, it is not clear how TAg perturbed the phosphorylation of p130 and p107. We explored the possibility that an in vitro approach would permit an examination of the effects of TAg on the phosphorylation of p130.

**EXPERIMENTAL PROCEDURES**

**Cell Culture—**T98G cells (ATCC; human glioblastoma multiforme cell line) were cultured in Dulbecco’s modified Eagle’s medium and fetal bovine serum (Invitrogen), 100 units/ml penicillin, and 100 \(\mu\)g/ml streptomycin in 10% CO\(_2\) at 37°C. To induce growth arrest, 2 \(\times\) 10\(^6\) cells were plated in a 150-mm dish and cultured in serum-free medium for 72 h. Cells were stimulated to reenter the cell cycle by addition of fresh medium containing 20% fetal bovine serum. The phase of the cell cycle was determined by fluorescence-activated cell sorter analysis of DNA content as measured by propidium iodide incorporation.

**Cell Extracts—**Cells were harvested by trypsin-EDTA and then...
washed twice with ice-cold phosphate-buffered saline and once with low salt extraction buffer (50 mM HEPES-NaOH (pH 7.4), 5 mM KCl, 1.5 mM MgCl₂, containing 1 mM dithiothreitol and Complete™ protease inhibitor (Roche Applied Science) (28). After supernatant was removed, cells were resuspended in residual buffer and sonicated. The lysate was centrifuged twice at 14,000 x g at 4 °C to remove cellular debris and stored in aliquots at −80 °C. For Western blot analysis, cells were washed twice with phosphate-buffered saline and lysed in extraction buffer C (50 mM Tris-HCl (pH 8.0), 125 mM NaCl, and 0.5% Nonidet P-40) containing 10 μM aprotinin, 10 μg/ml leupeptin, 0.1 mM phenylmethylsulfonyl fluoride, and 4 mM NaF and 0.1 mM Na₃VO₄.

Antibodies—p130 was detected with specific rabbit polyclonal antibody (antibody C-20, Santa Cruz Biotechnology). Additional antibodies used for blotting were anti-cyclin B (CB169, Upstate Biotechnology, Inc.) and anti p27kip1 (Ab-2, Neomarkers).

In Vitro Translation and Translation—In vitro translated (IVT) samples were generated in a coupled transcription and translation system using reticulocyte lysate according to the manufacturers’ recommendations (TNT; Promega, Madison, WI, and [35S]methionine; PerkinElmer Life Sciences). HA-p130 and HA-pRb in pCDNA3 were used as templates (12). The p130 phosphorylation mutants, p130WT and p130H42Q, were treated similarly (10). In vitro translation products were stored at −80 °C for up to 1 month prior to use.

Expression and Purification of T Ag—SV40 large T Ag fragments were expressed as glutathione S-transferase fusion proteins in the DH5α strain of Escherichia coli. T1–135 comprised the N-terminal 135 residues, and HQ1–135 contained a point substitution mutation H42Q that perturbed the DNA J domain. A 1-liter culture of bacteria containing pGEX2T-T1–135 or pGEX2T-HQ1–135 was collected in 1-ml fractions. Peak protein fractions were pooled and stored at −80 °C.

RESULTS

p130 Is Phosphorylated in Vitro in a Cell Cycle-specific Manner—T98G cells were cultured without serum for 72 h and then stimulated to reenter the cell cycle by addition of medium containing 20% serum. Cells were harvested 5 h and 24 h after serum addition and processed for fluorescence-activated cell scan analysis (A) or Western blotting (B). Lysates were separated in 6% (top panel), 10% (middle), and 12% (bottom) SDS-PAGE and immobiloblotted for p130, cyclin B (Cyc B), and p27kip1 (p27) as indicated. In panel B, the arrows represent phosphoforms 1–3 at p130.

In vitro translation products were stored at −80 °C for up to 1 month prior to use.

Expression and Purification of T Ag—SV40 large T Ag fragments were expressed as glutathione S-transferase fusion proteins in the DH5α strain of Escherichia coli. T1–135 comprised the N-terminal 135 residues, and HQ1–135 contained a point substitution mutation H42Q that perturbed the DNA J domain. A 1-liter culture of bacteria containing pGEX2T-T1–135 or pGEX2T-HQ1–135 was collected in 1-ml fractions. Peak protein fractions were pooled and stored at −80 °C.

RESULTS

p130 Is Phosphorylated in Vitro in a Cell Cycle-specific Manner—T98G cells were cultured without serum for 72 h and then stimulated to reenter the cell cycle by addition of medium containing 20% serum. Cells were harvested 5 h and 24 h after serum addition and processed for fluorescence-activated cell scan analysis (A) or Western blotting (B). Lysates were separated in 6% (top panel), 10% (middle), and 12% (bottom) SDS-PAGE and immobiloblotted for p130, cyclin B (Cyc B), and p27kip1 (p27) as indicated. In panel B, the arrows represent phosphoforms 1–3 at p130.
phosphorylation whether an intact T Ag J domain was required to perturb p130 compared the activity of T1–135 to determine but not the J domain mutant HQ1–135 type T1–135 but not the J domain mutant HQ1–135 could specifically co-immunoprecipitate IVT p130 since they both contain an intact LXCXE motif (data not shown).

IVT p130 appeared as a doublet, when incubated in the G1 lysates, that did not change when incubated with T1–135 (Fig. 3A, top panel). In contrast, addition of T1–135 to the S phase lysates reduced the relative amount of hyperphosphorylated IVT p130 (Fig. 3A, second panel). To confirm that IVT p130 underwent specific phosphorylation when incubated in S phase lysates, we examined the ability of certain p130 mutants to become phosphorylated when mixed with S phase lysates. At least 22 residues within p130 are known to be phosphorylated in vivo (7, 10). Cyclin D1/Cdk4, cyclin E/Cdk2, and cyclin A/Cdk2 contribute to the phosphorylation of p130 on specific serine and threonine residues. Cdk4-dependent phosphorylation sites within p130 were identified by determining the sensitivity of a given residue to phosphorylation in the presence of the Cdk4-inhibitor p16INK4A (10). The ΔCdk4 allele substitutes the Cdk4-dependent phosphorylation sites with alanine (T401A, S672A, and S1035A). Similar to wild type p130, IVT ΔCdk4 appeared initially as a doublet that became rapidly hyperphosphorylated when incubated in S phase extracts (Fig. 3A, third panel). Addition of T1–135 to the mixture reduced the hyperphosphorylated forms of ΔCdk4. The PM19A allele contains alanine substitutions of 19 Cdk-dependent phosphorylation sites and when in vitro translated, it appeared as a single band that changed minimally in gel mobility when incubated with S phase lysates (Fig. 3A, bottom panel). Addition of T1–135 did not affect the gel migration pattern of the mutant PM19A. Taken together, these results suggest that IVT p130 was specifically phosphorylated when incubated in S phase extracts and that T Ag reduced the phosphorylation of p130 under these conditions.

The ability of SV40 T Ag to perturb the phosphorylation of p130 in vivo requires an intact DNA J domain (24, 27). We compared the activity of T1–135 with HQ1–135 to determine whether an intact T Ag J domain was required to perturb p130 phosphorylation in vitro. As shown in Fig. 3B (top panel), wild type T1–135 but not the J domain mutant HQ1–135 reduced the phosphorylation of IVT p130 when incubated in S phase extracts. Indeed, the overall phosphorylation state of p130 appeared to be enhanced when incubated with HQ1–135. The ability of J domain mutant T Ag to enhance the phosphorylation of p130 has also been observed in vivo (24).

Although wild type T Ag can affect the phosphorylation of p130 and p107, it has no apparent effect on pRb phosphorylation in vivo (24, 26, 27, 32, 33). To determine whether the ability of T Ag to perturb p130 phosphorylation in vitro was specific, we compared the ability of wild type and J domain mutant T Ag to affect the phosphorylation of pRb. Similar to p130, incubation of IVT pRb with S phase lysates led to a rapid change in gel mobility (Fig. 3B, bottom panel) that was sensitive to λ-PPase (data not shown), suggesting that pRb was also specifically phosphorylated in vitro. However, addition of wild type T1–135 or the J domain mutant HQ1–135 had no effect on the phosphorylation of pRb (Fig. 3B). The inability of T Ag to perturb pRb phosphorylation may reflect the inability of T Ag to bind to phosphorylated pRb (33).

We considered the possibility that T Ag reduced the overall level of p130 phosphorylation by promoting the degradation of the hyperphosphorylated forms of p130. However, the amount of IVT p130 did not decrease after addition of T1–135 when incubated in S phase lysates when quantitated by phosphorimaging. Furthermore, addition of the proteasome inhibitor lactacystin did not affect the ability of T1–135 to reduce the gel migration of IVT p130 in S phase lysates (Fig. 3C).

**SV40 T Ag Effect on p130 Phosphorylation Is Dependent on a Phosphatase Activity**—The effect of T Ag on the phosphorylation of IVT p130 may be secondary to inhibition of a kinase or recruitment of a phosphatase. To distinguish between these possibilities, IVT p130 was incubated with S phase lysates prior to addition of T Ag. After a 1-h incubation in S phase lysates, IVT p130 became hyperphosphorylated as expected (Fig. 4A, lane 2). The reaction was subsequently aliquoted and incubated for an additional 2 h alone (lane 3) or with T1–135 (lane 4) and HQ1–135 (lane 5). Phosphorylation of p130 was significantly reduced when T was added after IVT p130 had become phosphorylated (lane 4). In contrast, IVT p130 remained hyperphosphorylated when incubated with HQ1–135. The ability of wild type T Ag to reduce the phosphorylation of p130 suggests that T Ag recruited a phosphatase to promote the dephosphorylation of p130.

The serine/threonine protein phosphatases PP1 and PP2A have been shown to be crucial in regulating RB activity. To determine whether p130 phosphorylation was sensitive to PP1 or PP2A, increasing concentrations of okadaic acid (OA) were incubated with S phase lysates and IVT p130 (34) (Fig. 4B). Addition of 0.1 or 1 μM OA led to a slight increase in the relative amount of hyperphosphorylated p130, suggesting that a phosphatase-specific for p130 was active in the S phase lysates. In the presence of no or 5 nM OA, T1–135 remained capable of reducing the phosphorylation of p130 (Fig. 4B, bottom panel).

To characterize the phosphatase activity recruited by T Ag, a comparison of inhibitors I-1 PP2A and I-2PP1 with OA was performed (34). IVT p130 was incubated in S phase lysates for 1 h to induce phosphorylation (Fig. 4C, lane 2). The reaction mixture was aliquoted and incubated with PP1-specific inhibitor.
and T1–135 for an additional 2 h. Although I-2PP1 did not block the ability of T1–135 to reduce p130 phosphorylation (lane 3), I-1PP2A partially inhibited the effect of T Ag on the phospho-fluorography. In B, IVT p130 (top panel) or pRb (bottom) was incubated with T1–135 (T, lane 4) or HQ1–135 (HQ, lane 5) for an additional 2 h. In C, OA was incubated with IVT p130 and S phase extracts (top panel) and in the presence of T1–135 (bottom panel). In C, IVT p130 was incubated in S phase extracts for 0 (lane 1) or 1 h (lane 2). After 1 h, the reaction was split into four aliquots with addition of PP1A inhibitor (10 nM), PP2A inhibitor (12 nM), OA (1 µM), and T1–135 as indicated and incubated for an additional 2 h at 30 °C.

Fig. 4. Large T Ag promotes dephosphorylation of p130. In A, IVT p130 was incubated in S phase extracts and an energy-regenerating system for 0 (lane 1) or 1 h (lane 2). After 1 h, the reaction was split into three aliquots and incubated alone (lane 3) or with addition of T1–135 (T, lane 4) or HQ1–135 (HQ, lane 5) for an additional 2 h. In B, OA was incubated with IVT p130 and S phase extracts (top panel) and in the presence of T1–135 (bottom panel). In C, IVT p130 was incubated in S phase extracts for 0 (lane 1) or 1 h (lane 2). After 1 h, the reaction was split into four aliquots with addition of PP1A inhibitor (10 nM), PP2A inhibitor (12 nM), OA (1 µM), and T1–135 as indicated and incubated for an additional 2 h at 30 °C.

(I-2PP1, lane 3) or PP2A-specific inhibitor (I-1PP2A, lanes 4–6) and T1–135 for an additional 2 h. Although I-2PP1 did not block the ability of T1–135 to reduce p130 phosphorylation (lane 3), I-1PP2A partially inhibited the effect of T Ag on the phospho-fluorography. In B, IVT p130 (top panel) or pRb (bottom) was incubated with T1–135 or HQ1–135 (HQ) as described in panel A for the indicated times. In C, IVT p130 was incubated in S phase extracts in the presence of lactacystin (Lacta) or solvent (Me2SO (DMSO)) and T1–135 or HQ1–135 for the indicated times. In D, purified T1–135 and HQ1–135 (1.0 µg) were separated in a 10% SDS-polyacrylamide gel and silver stained. Molecular size (MS) standards are indicated.

---

Fig. 3. Large T Ag alters p130 phosphorylation in S phase extracts. In A, IVT p130 was incubated in the presence or absence of purified T1–135 (T) in G1 (top panel) or S phase (second panel) extracts and an energy-regenerating system at 30 °C for the indicated times. The p130 phosphorylation mutants ∆Cdk4 and PM19A were incubated in S phase extracts and treated in a similar manner. Reactions were stopped with SDS buffer, resolved in 6% SDS-PAGE, and detected by fluorography. In B, IVT p130 (top panel) or pRb (bottom) was incubated with T1–135 or HQ1–135 (HQ) as described in panel A for the indicated times. In C, IVT p130 was incubated in S phase extracts in the presence of lactacystin (Lacta) or solvent (Me2SO (DMSO)) and T1–135 or HQ1–135 for the indicated times. In D, purified T1–135 and HQ1–135 (1.0 µg) were separated in a 10% SDS-polyacrylamide gel and silver stained. Molecular size (MS) standards are indicated.
rylation of p130 (lane 4). Addition of OA with I-1PP2A inhibited the ability of T1–135 to reduce p130 phosphorylation (lanes 5 and 6). These results suggest the role of a PP2A-specific activity as well as perhaps additional phosphatase activities in the T Ag-mediated dephosphorylation of p130.

**DISCUSSION**

The study of viral oncoproteins continues to provide insights into the regulation of the cell cycle by the Rb family. Previous work had established that the LXCXE motif and J domain of T Ag cooperate to inactivate the growth suppression and E2F repression functions of p130 (24, 26). These domains of T Ag were required to promote the release of E2F4 from p130 in gel mobility shift assays and to perturb p130 phosphorylation (24–27). To determine how T Ag affected p130 phosphorylation, an in vitro assay was developed. We observed that lysates prepared from S phase enriched cells supported the specific phosphorylation of p130. Addition of T Ag led to a dephosphorylation of p130 in a J domain-dependent manner.

Our evidence suggests that SV40 large T Ag recruits a serine/threonine phosphatase to promote the dephosphorylation of p130 given that OA completely inhibited the effect of T Ag. OA has a 4,000-fold lower Kᵢ for PP2A than PP1 (35). Given the relatively high concentrations of OA (≥100 nM) required to inhibit the effect of T Ag on p130 phosphorylation, a PP2A-like phosphatase activity was likely involved. Use of OA may be particularly informative when studied in vitro because OA has profound effects on cellular physiology when added in vivo. For example, OA treatment of NIH 3T3 cells results in a G1 arrest and decreased pRb phosphorylation, decreased Cdk activity, and a reduction in cyclin A, Cdc2, and Cdk2 expression (36). Furthermore, OA is relatively PP2A selective in vitro but may indirectly inhibit PP1 due to secondary accumulation of I-1PP1 (34). We expect this effect to be minimized in vitro. Notably, OA can inhibit other members of the protein phosphatase family, including PP4 and PP5, so the contribution of additional serine/threonine phosphatases cannot be ruled out (34, 37).

The ability of I-1PP2A to at least partially inhibit the effect of T Ag on p130 phosphorylation also supports a role for PP2A. At inhibitory concentrations, I-1PP2A partially reverses T Ag-mediated dephosphorylation of p130. The remaining effect of T Ag was reversed by addition of OA together with I-1PP2A. Although I-1PP1, a PP1 protein-specific inhibitor, did not reverse T Ag-mediated dephosphorylation of p130, we cannot rule out that PP1 was not also involved.

PP2A is an important negative regulator of mitogenic activity that serves to dephosphorylate and activate key kinases such as MAP kinase and MAP kinase kinase. PP2A is a family of serine-threonine phosphatases consisting of two regulatory subunits, A and B, and a catalytic subunit C. Notably, SV40 small T Ag can bind specifically to PP2A by binding to the A and C subunit of PP2A and displacing the B subunit (38). SV40 small T Ag can inhibit PP2A activity, resulting in activation of the MAP kinase pathway and promotion of cell growth (39, 40). Expression of small T antigen can enhance SV40 large T Ag transformation activity and is required for SV40 transformation of human fibroblasts (41, 42). Although small T Ag shares the N-terminal J domain with large T Ag, the small T J domain is not necessary for binding to PP2A (40). The small T antigen J domain may participate in regulation of small T antigen-PP2A complex (43). Of note, small T antigen was not present in any of these assays, nor did the large T Ag constructs encode for small T antigen.

There is considerable evidence suggesting that phosphatases regulate the RB family members during the normal cell cycle and in response to stress conditions. PP1 has been shown to directly dephosphorylate pRb during mitosis, whereas PP2A may be indirectly involved in the pRb pathway by regulating G1 Cdk activity (44–46). A catalytic subunit PP1p102 was found to bind pRb in a yeast two-hybrid assay and could associate with pRb during mitosis and early G1 (47). During hypoxia-induced arrest, pRb becomes rapidly dephosphorylated due to an increased pRb-directed phosphatase activity and inhibition of Cdk4 and Cdk2 activity (48). In response to UV irradiation, p107 will undergo rapid dephosphorylation by a PP2A-like activity (49). In addition, p107 binds to PR59, a regulatory B subunit of PP2A (50).

Adenovirus E1A can also block hyperphosphorylation of p130 and p107 (51). The reported mechanism includes inhibition of Cdk-phosphorylation that can be overcome by overexpression of D-type cyclins (52). Since both E1A and T Ag have developed a mechanism to reduce the relative amounts of hyperphosphorylated p130, it suggests the possibility that hyperphosphorylated p130 may have specific roles in growth regulation that the viral oncoproteins target for inactivation.

It would be interesting to speculate why the J domain of large T Ag may specifically recruit a phosphatase activity. T Ag itself is modified by phosphorylation, and certain residues affect T Ag-dependent viral DNA replication. For example, phosphorylation of T Ag residue Thr-124 is required for SV40 viral DNA replication, whereas phosphorylation of residues Ser-120 and Ser-123 inhibits T Ag replication activity, perhaps by inhibiting the phosphorylation of Thr-124 (53, 54). Therefore, dephosphorylation of Ser-120 and Ser-123 may promote T Ag replication, and this has been reported to be dependent on PP2A activity (55–60). Furthermore, dephosphorylation of Ser-120 and Ser-123 by PP2A can be inhibited by SV40 small T antigen (61). In addition, it has been reported that large T Ag itself can be dephosphorylated by incubation with S phase extracts (62). The N-terminal J domain of large T Ag contributes to SV40 replication in vitro, although the mechanism has not been clarified (22). Perhaps the large T Ag J domain serves to recruit a phosphatase to dephosphorylate T Ag residues Ser-120 and Ser-123, thereby promoting phosphorylation of Thr-124 and efficient viral DNA replication. The large T Ag J domain-associated phosphatase would serve two potential functions including dephosphorylation of T Ag to promote viral replication and dephosphorylation of p130 to promote transformation.

**Acknowledgments**—We thank Holgar Bastians and Joan Ruderman for guidance with the in vitro assay, Hiroshi Matsuo for purification of T Ag, and Jiri Bartek for plasmids.

**REFERENCES**

1. DeCaprio, J. A., Ludlow, J. W., Lynch, D., Furukawa, Y., Griffin, J., Pwnica-Worms, H., Huang, C-M., and Livingston, D. C. (1989) Cell 58, 1085–1095.
2. Beijersbergen, R. L., Carleé, L., Kerhoven, R. M., and Bernards, R. (1995) Genes Dev. 9, 1540–1553.
3. Lundberg, A. S., and Weinberg, R. A. (1998) Mol. Cell. Biol. 18, 753–761.
4. Ewen, M. E., Sluss, H. K., Sherr, C. J., Matsuutome, H., Kato, J-Y., and Weinberg, R. A. (1995) Cell 73, 487–497.
5. Dowdy, S. F., Hinds, P. W., Louie, K., Reed, S. I., Arnold, A., and Weinberg, R. A. (1993) Cell 73, 499–511.
6. Connell-Crowley, L., Harper, J. W., and Goodrich, D. W. (1997) Mol. Cell. Biol. 17, 287–301.
7. Canhoto, A. J., Chestukhin, A., Litovchick, L., and DeCaprio, J. A. (2000) Oncogene 19, 5116–5122.
8. Mayol, X., Garriga, J., and Grana, X. (1995) Oncogene 11, 801–808.
9. Mayol, X., Garriga, J., and Grana, X. (1996) Oncogene 13, 237–246.
10. Hansen, K., Farkas, T., Lukas, J., Holm, K., Ronstrand, L., and Bartek, J. (2001) EMBO J. 20, 422–432.
11. Farkas, T., Hansen, K., Holm, K., Lukas, J., and Bartek, J. (2002) J. Biol. Chem. 277, 26741–26752.
12. Chestukhin, A., Litovchick, L., Rudich, K., and DeCaprio, J. A. (2002) Mol. Cell. Biol. 22, 453–468.
13. Tedesco, D., Lukas, J., and Reed, S. I. (2002) Genes Dev. 16, 2946–2957.
14. Smith, E. J., Leone, G., and Nevins, J. R. (1998) Cell Growth Differ. 9, 297–303.
15. Smith, E. J., Leone, G., DeGregori, J., Jakoi, L., and Nevins, J. R. (1996) Mol. Cell. Biol. 16, 6965–6976.
16. DeCaprio, J. A., Ludlow, J. W., Figge, J., Shew, J.-Y., Huang, C.-M., Lee, W.-H., Marsilio, E., Paucha, E., and Livingston, D. M. (1988) *Cell* **54**, 275–283
17. Zalvide, J., and DeCaprio, J. A. (1995) *Mol. Cell. Biol.* **15**, 5800–5810
18. Kelley, W. L., and Landry, S. J. (1994) *Trends Biochem. Sci.* **19**, 277–278
19. Peden, K. W., and Pipas, J. M. (1992) *Virus Genes* **6**, 107–118
20. Sawai, E. T., and Butel, J. S. (1989) *Mol. Cell. Biol.* **9**, 3422–3430
21. Sawai, E. T., Rasmussen, G., and Butel, J. S. (1994) *Virus Res.* **29**, 367–378
22. Campbell, K. S., Mullane, K. P., Aksoy, I. A., Stubdal, H., Zalvide, J., Pipas, J. M., Silver, P. A., Roberts, T. M., Schaffhausen, B. S., and DeCaprio, J. A. (1997) *Genes Dev.* **11**, 1098–1110
23. Srinivasan, A., McClellan, A. J., Vartikar, J., Marks, I., Cantalupo, P., Li, Y., Whyte, P., Rundell, K., Brodsky, J. L., and Pipas, J. M. (1997) *Mol. Cell. Biol.* **17**, 4761–4773
24. Stubdal, H., Zalvide, J., Campbell, K. S., Schweitzer, C., Roberts, T. M., and DeCaprio, J. A. (1997) *Mol. Cell. Biol.* **17**, 4979–4990
25. Sullivan, C. S., Cantalupo, P., and Pipas, J. M. (2000) *Mol. Cell. Biol.* **20**, 6233–6243
26. Zalvide, J., Stubdal, H., and DeCaprio, J. A. (1998) *Mol. Cell. Biol.* **18**, 1408–1415
27. Stubdal, H., Zalvide, J., and DeCaprio, J. A. (1996) *J. Virol.* **70**, 2781–2788
28. Bastians, H., Townsley, F. M., and Ruderman, J. V. (1998) *Proc. Natl. Acad. Sci. U. S. A.* **95**, 15374–15381
29. Bastians, H., Topper, L. M., Gorbsky, G. L., and Ruderman, J. V. (1999) *Mol. Biol. Cell* **10**, 3927–3941
30. Brandeis, M., and Hunt, T. (1996) *EMBO J.* **15**, 5280–5289
31. Chen, P. L., Scully, P., Shew, J. Y., Wang, J. Y., and Lee, W. H. (1989) *Cell* **58**, 1193–1198
32. Chao, H. H., Buchmann, A. M., and DeCaprio, J. A. (2000) *Mol. Cell. Biol.* **20**, 7624–7633
33. Ludlow, J. W., DeCaprio, J. A., Huang, C. M., Lee, W. H., Paucha, E., and Livingston, D. M. (1989) *Cell* **56**, 57–65
34. Shepecek, J. E., 2nd, Gauss, C. M., and Chamberlin, A. R. (1997) *Bioorg. Med. Chem.* **5**, 1739–1750
35. Takai, A., Sasaki, K., Nagai, H., Mieskes, G., Isobe, M., Isono, K., and Yoshimoto, T. (1995) *Biochem. J.* **306**(Pt 3), 657–665
36. Schonthal, A., and Peramiesco, J. R. (1993) *Oncogene* **8**, 433–441
37. Cohen, P., Holmes, C. F., and Tsukitani, Y. (1990) *Trends Biochem. Sci.* **15**, 98–102
38. Pallarès, D. C., Shahrik, I. K., Martin, B. L., Jaspers, S., Miller, T. B., Brautigan, D. L., and Roberts, T. M. (1990) *Cell* **60**, 167–176
39. Yang, S. I., Lickteig, R. L., Estes, R., Rundell, K., Walter, G., and Mumbery, M. C. (1991) *Mol. Cell. Biol.* **11**, 1988–1995
40. Sontag, E., Fedorov, S., Kamihayashi, C., Robbins, D., Cobb, M., and Mumbery, M. (1993) *Cell* **75**, 887–897
41. Bikel, I., Montano, X., Agba, M. E., Brown, M., McCormack, M., Boltax, J., and Livingston, D. M. (1987) *Cell* **48**, 321–330
42. Hahn, W. C., Dessain, S. K., Brooks, M. W., King, J. E., Elenbaas, B., Sabatini, D. M., DeCaprio, J. A., and Weinberg, R. A. (2002) *Mol. Cell. Biol.* **22**, 2111–2123
43. Matee, S. C., Fedorov, S. A., and Mumbery, M. C. (1998) *J. Biol. Chem.* **273**, 35339–35346
44. Yan, Y., and Mumbery, M. C. (1999) *J. Biol. Chem.* **274**, 31917–31924
45. Nelson, D. A., and Ludlow, J. W. (1997) *Oncogene* **14**, 2407–2415
46. Nelson, D. A., Krucher, N. A., and Ludlow, J. W. (1997) *J. Biol. Chem.* **272**, 4528–4535
47. Durfee, T., Becherer, K., Chen, P. L., Yeh, S. H., Yang, Y., Kilburn, A. E., Lee, W. H., and Eldridge, S. J. (1993) *Genes Dev.* **7**, 555–569
48. Krtolica, A., Krucher, N. A., and Ludlow, J. W. (1998) *Oncogene* **17**, 2285–2304
49. Voorhoeve, P. M., Watson, R. J., Farlie, P. G., Bernards, R., and Lam, E. W. (1999) *Oncogene* **18**, 679–688
50. Voorhoeve, P. M., Hjmanis, E. M., and Bernards, R. (1999) *Oncogene* **18**, 515–524
51. Parreno, M., Garriga, J., Limon, A., Mayol, X., Beck, G. R., Jr., Morán, E., and Grana, X. (2000) *J. Virol.* **74**, 3166–3176
52. Parreno, M., Garriga, J., Limon, A., Albrecht, J. H., and Grana, X. (2001) *Oncogene* **20**, 4783–4806
53. Cegielska, A., Moarefi, I., Fanning, E., and Virshup, D. M. (1994) *J. Virol.* **68**, 269–275
54. Grasser, F. A., Scheidtmann, K. H., Trauern, P. T., Traug, J. A., and Walter, G. (1988) *Virology* **165**, 13–22
55. Cegielska, A., Shaffner, S., Derau, R., Goria, J., and Virshup, D. M. (1994) *Mol. Cell. Biol.* **14**, 4616–4623
56. Cegielska, A., and Virshup, D. M. (1993) *Mol. Cell. Biol.* **13**, 1292–1211
57. Virshup, D. M., Kaufman, M. G., and Kelly, T. J. (1989) *EMBO J.* **8**, 3091–3098
58. Virshup, D. M., Ross, A. A., and Kelly, T. J. (1992) *Mol. Cell. Biol.* **12**, 4883–4895
59. Scheidtmann, K. H., Virshup, D. M., and Kelly, T. J. (1991) *J. Virol.* **65**, 2098–2101
60. Lawson, R., Cohen, P., and Lane, D. P. (1996) *J. Virol.* **64**, 2380–2383
61. Scheidtmann, K. H., Mumbery, M. C., Rundell, K., and Walter, G. (1991) *Mol. Cell. Biol.* **11**, 2006–2009
62. Ludlow, J. W. (1992) *Oncogene* **7**, 1011–1014
