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Transcriptional Repression by the Rb-Related Protein p107

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The transcription factor DRTF1/E2F is believed to play an important role in regulating cellular proliferation because it undergoes a series of periodic interactions with proteins that are known to be important regulators of the cell cycle, including the retinoblastoma gene product (pRb) and cyclin A. Furthermore, certain viral oncogene products, such as adenovirus E1a, disrupt these DRTF1/E2F complexes by sequestering the associated proteins. p107, a protein that is structurally related to pRb, also binds to DRTF1/E2F, and in this study we investigate the functional consequences of this interaction. We show that p107 can repress E2F binding site-dependent transcription and that the adenovirus E1a protein overcomes p107-mediated transcriptional repression. Two distinct but related proteins, pRb and p107, can therefore repress transcription driven by DRTF1/E2F, whereas the E1a protein overrides the repression. We also demonstrate that the transcription repressing properties of p107 and pRb are influenced by the cell type and by differentiation, because neither protein affects transcription in F9 embryonal carcinoma (EC) cells but both do so efficiently in differentiated derivatives. In this respect, the repressing activities of pRb and p107 inversely correlate with the presence of the cellular E1a-like activity previously documented in F9 EC cells. These data suggest that p107 and pRb exert their biological activities in some but not all cell types.

INTRODUCTION

It is believed that the cellular transcription factor DRTF1/E2F plays an important role in integrating cell-cycle events with the transcription apparatus because during cell-cycle progression in mammalian cells, it undergoes a series of periodic interactions with molecules that are known to be important regulators of cellular proliferation. For example, the retinoblastoma tumor suppressor gene product (pRb), which negatively regulates progression from G1 into S phase and is frequently modified in tumor cells (Horowitz *et al.*, 1990; Hu *et al.*, 1990; Huang *et al.*, 1990), binds to DRTF1/E2F (Bandara and La Thangue, 1991; Chellapan *et al.*, 1991; Chittenden *et al.*, 1991). Similarly, the pRb-related protein p107 binds to DRTF1/E2F to produce a complex that predominates during S phase (Devoto *et al.*, 1992; Shirodkar *et al.*, 1992; Bandara *et al.*, 1993).

The interaction between pRb and DRTF1/E2F leads to repression of E2F site-dependent transcription (Hiebert *et al.*, 1992; Zamanian and La Thangue, 1992). This is likely to be important in mediating the growth suppressing activity of pRb because E2F binding sites occur in the transcriptional control regions of a variety of genes, the protein products of which are necessary for cellular proliferation (Blake and Azizkhan, 1989; Pearson *et al.*, 1991). For example, c-*myc*, *DHFR*, and $p34^{cdc2}$ have functional E2F binding sites in their promoters (Hiebert *et al.*, 1989; Hiebert *et al.*, 1991; Dalton, 1992), and transcriptional repression of these genes could therefore be the basis of Rb-mediated growth control.

Further support for this idea stems from observations made on the proteins encoded by naturally occurring mutant *Rb* alleles, which fail to bind to DRTF1/E2F (Bandara *et al.*, 1991; Hiebert *et al.*, 1992) and are unable to repress E2F site-dependent transcription (Hiebert *et al.*, 1992; Zamanian and La Thangue, 1992). Such mutant Rb proteins would be predicted to lack the capacity to negatively regulate proliferation and hence their inactivation could contribute to the escape from growth control that is characteristic of tumor cells.

In addition, certain viral oncoproteins, notably adenovirus E1a, SV40 large T antigen, and the human papilloma virus E7 protein, modulate DRTF1/E2F by se-

questering pRb and other associated proteins (Bandara and La Thangue, 1991; Shirodkar *et al.*, 1992; Morris *et al.*, 1993). This property allows E1a to overcome the repression of E2F site-dependent transcription by pRb (Hiebert *et al.*, 1992; Zamanian and La Thangue, 1992) and thus may contribute to the diverse biological activities associated with this protein (Lillie *et al.*, 1987; Moran and Mathews, 1987). Importantly, this effect requires regions in the E1a protein that are necessary for cellular transformation of tissue culture cells and hence to overcome growth control (Lillie *et al.*, 1987).

The biological activities of p107 have not been explored so far. Moreover, unlike the situation for pRb, the functional consequences of the interaction between p107 and DRTF1/E2F have not been easy to predict because during cell-cycle progression, the p107-DRTF1/E2F complex correlates with the transcriptional induction of some genes that contain DRTF1/E2F binding sites, suggesting that the p107-DRTF1/E2F complex is capable of activating transcription. We therefore undertook a study to determine the functional consequences of the interaction between p107 and DRTF1/E2F.

We show here for the first time that p107 represses transcription driven by the E2F binding site and furthermore that viral oncoproteins, such as E1a, overcome this repression. We also demonstrate that the ability of pRb and p107 to repress transcription is influenced by the cell type and the process of differentiation. We believe that these observations have important implications for understanding the role of pRb and p107 during tumorigenesis and differentiation.

MATERIALS AND METHODS

Cell Culture

F9 embryonal carcinoma (EC) and SAOS-2 cells were maintained as adherent monolayers in Dulbecco's modified Eagle's medium supplemented with 10% heat-inactivated fetal calf serum. Cells were replated at a density of $\sim 5 \times 10^4$ per ml (F9 EC) or 1×10^5 per ml (SAOS-2) every 3 and 4 d, respectively. F9 EC cells were differentiated by treatment with 5×10^{-8} M all *trans* retinoic acid, 10^{-3} M dibutyryladenosine 3',5' monophosphate, and 10^{-4} M isobutylmethylxanthine as previously described (Tassios and La Thangue, 1990).

Plasmids

E2F site-dependent reporters, Rb expression vectors, and pJ3Ω12S were as previously described (Zamanian and La Thangue, 1992). For pCMV107S and pCMV107AS, a human cDNA encoding an open reading frame of 936 amino acids and derived from plasmid λZap H p107c (Ewen *et al.*, 1991) was cloned either in the sense (S) or antisense (AS) orientation downstream of an artificial translation initiation sequence. The predicted DNA sequence was confirmed by DNA sequencing.

Transient Transfection

Cells were transfected by the calcium phosphate coprecipitate procedure as previously described (Zamanian and La Thangue, 1992). The amounts of each construct used per transfection are detailed in the figure legends. For each transfection pBluescript SK was included to maintain the final DNA concentration constant. All transfections included an internal control (pCMV-βgal) (Tassios and La Thangue, 1990). The assay for computer-aided tomography activity and quantitation of thin-layer chromatography plates have been described before (Zamanian and La Thangue, 1992).

Cell Staining for β-Galactosidase

Cells were transfected as described in Figure 4 and fixed in 0.5% glutaraldehyde 42 h after transfection, after which they were stained overnight for β-galactosidase activity as described by Dannenberg and Suga (1981).

Immunofluorescence

Immunofluorescence was performed as previously described (La Thangue, 1984) after fixing cells for 2 min at room temperature in methanol/acetone (1:1).

RESULTS

To assess the effect of pRb and p107 on the transcriptional activity of DRTF1/E2F, we prepared a panel of reporter constructs in which three wild-type (p3xWT) or mutant (p3xMT) E2F binding sites, taken from the adenovirus E2A promoter (La Thangue *et al.*, 1990) and placed upstream of the minimal herpes simplex virus thymidine kinase promoter, direct the expression of the chloramphenicol acetyl transferase (cat) gene (Figure 1). Effector constructs carrying either a cDNA encoding wild-type human pRb or a cDNA derived from a naturally occurring mutant *Rb* allele lacking exon 22 (HRbΔ22), which served as a negative control (Zamanian and La Thangue, 1992), were used to examine the effect of pRb on the activity of DRTF1/E2F. To investigate the properties of p107, a wild-type human coding sequence (Ewen *et al.*, 1991) was cloned in either the S or AS orientation in the same expression vector backbone (Figure 1).

p107 Represses E2F Site-Dependent Transcription in SAOS-2 Cells

We first determined the effect of expressing p107 on the activity of p3xWT in SAOS-2 cells. These cells contain a mutated *Rb* allele, and we previously have shown that introduction of wild-type pRb correlates with repression of E2F site-dependent transcription (Zamanian and La Thangue, 1992). Similarly, we chose to study the activity of p107 by increasing the levels of p107 expressed in SAOS-2 cells by transfection of the pCMV107S expression vector. Thus, pCMV107S, but not pCMV107AS, caused a significant reduction in the transcriptional activity of p3xWT (Figure 2A; compare lanes 1 and 2 with 7–10). As expected from our previous studies, wild-type pRb repressed the activity of p3xWT, whereas HRbΔ22 did not (Figure 2A; compare lanes 1 and 2 with 3–6). Repression by p107 was specific for E2F site-dependent transcription because neither the

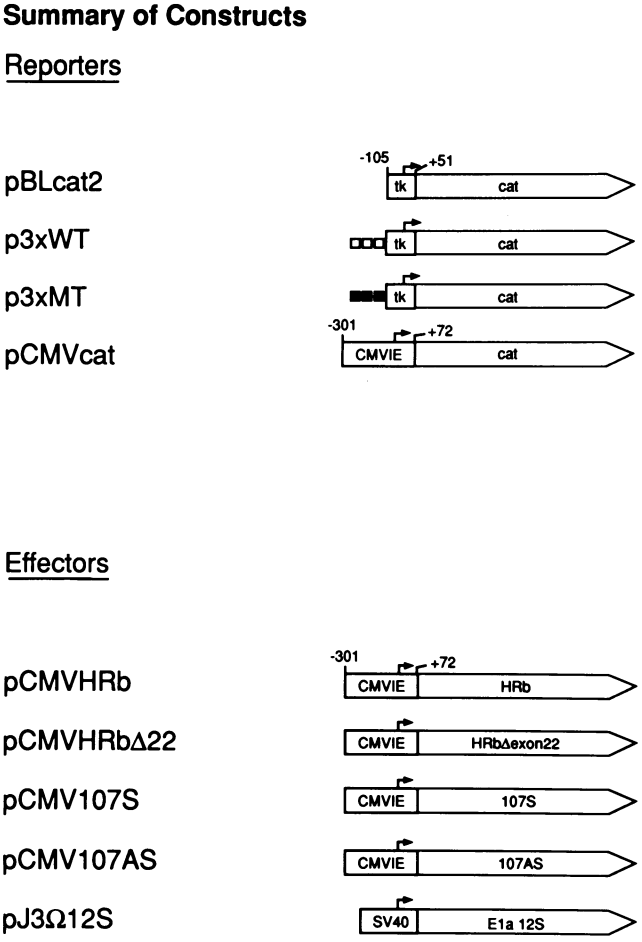

Figure 1. Summary of constructs. Reporter constructs were all derived from pBLcat2 and have been previously described (Zamanian and La Thangue, 1992). The wild-type (WT) E2F binding site was taken from the −71 to −50 region of the adenovirus E2A promoter, and the mutant (MT) binding site is mutated in nucleotides −62 to −60 (La Thangue *et al.*, 1990). Open and solid boxes denote wild-type and mutant E2F binding sites, respectively. pCMVcat has been described previously (Tassios and La Thangue, 1990). pRb expression vectors were generated by replacing the coding sequence in pCMVcat with a wild-type or mutant human Rb cDNA deleted in exon 22, creating pCMVHRb and pCMVHRbΔ22, respectively (Zamanian and La Thangue, 1992). For pCMV107S and pCMV107AS, a human cDNA containing an open reading frame of 936 amino acid residues (Ewen *et al.*, 1991) was cloned either in the sense (S) or antisense (AS) orientation downstream of an artificial translation initiation sequence. The predicted DNA sequence was confirmed by DNA sequencing. E1A expression vector pJ3Ω12S, which contains a 12S cDNA, has been described previously (Zamanian and La Thangue, 1992).

activity of p3xMT nor pCMVcat was affected by the increased level of p107 (Figure 2A).

We next investigated whether the repressing effect of p107 correlates with the level of p107. Increasing amounts of either pCMV107S or pCMV107AS were therefore transfected into SAOS-2 cells and the effect on the transcriptional activity of p3xWT assessed. As the amount of pCMV107S increased, there was a concomitant decline in the activity of p3xWT (Figure 2B; lanes 1 to 10), whereas a similar titration performed with pCMV107AS had no apparent effect (Figure 2B; compare lanes 1 and 2 with 11 through 18). We conclude that in SAOS-2 cells, repression of E2F site-dependent transcription correlates with the level of p107 and therefore that both pRb and p107 negatively regulate transcription driven by the E2F binding site.

The Repressing Properties of p107 and pRb Are Differentiation Regulated

To test if the effects of p107 and pRb on E2F site-dependent transcription are influenced by the cell type, we studied the transcription-regulating properties in F9 EC cells, the stem cells of teratocarcinomas, and their parietal endoderm (PE)-like differentiated derivatives. We chose to study F9 EC cells because they are thought to resemble cells in the early mammalian embryo (Hogan *et al.*, 1981), and thus studies in these cells are likely to be relevant for understanding the regulation of E2F site-dependent transcription during embryogenesis.

The E2F sites in p3xWT are efficient upstream activating sequences in F9 EC cells (Zamanian and La Thangue, 1992), where they activate transcription about seven-fold more efficiently than in F9 PE cells. This correlates with the high levels of transcriptionally active DRTF1 in these cells (La Thangue and Rigby, 1987; La Thangue *et al.*, 1990). Transfection of either pCMVHRb or pCMV107S into F9 EC cells, however, did not significantly affect the activity of p3xWT (Figure 3A). To rule out trivial explanations for this phenotype, we confirmed that the CMV sequences that drive the expression of pRb and p107 were active in F9 EC cells and also determined the transfection efficiency of F9 EC cells using pCMV-βgal (Tassios and La Thangue, 1990). β-Galactosidase activity was detectable in ~10% of the cells in a culture of F9 EC cells (a typical example is shown in Figure 4A), indicating that the CMV sequences were transcriptionally active in this cell type. This transfection efficiency is similar to the efficiency of other types of cells, including differentiated F9 cells (see below). Furthermore, we confirmed that pRb encoded by pCMVHRb was synthesized in F9 EC cells using a monoclonal antibody that recognizes human pRb, IF8 (Bartek *et al.*, 1992), which stained a similar proportion of cells (Figure 4C; ~10%) to those which expressed β-galactosidase from pCMV-βgal (Figure 4A). Thus, the lack of repression by p107 and pRb is unlikely to be due to inefficient expression of either protein.

However, both p107 and pRb were able to repress E2F site-dependent transcription in F9 cells after they had been induced to differentiate. This required wild-type pRb and p107 coding sequence because HRbΔ22 and 107AS effectors failed to have any significant effect on the activity of p3xWT (Figure 3B). This repression was also dependent on the amount of the pCMV107S

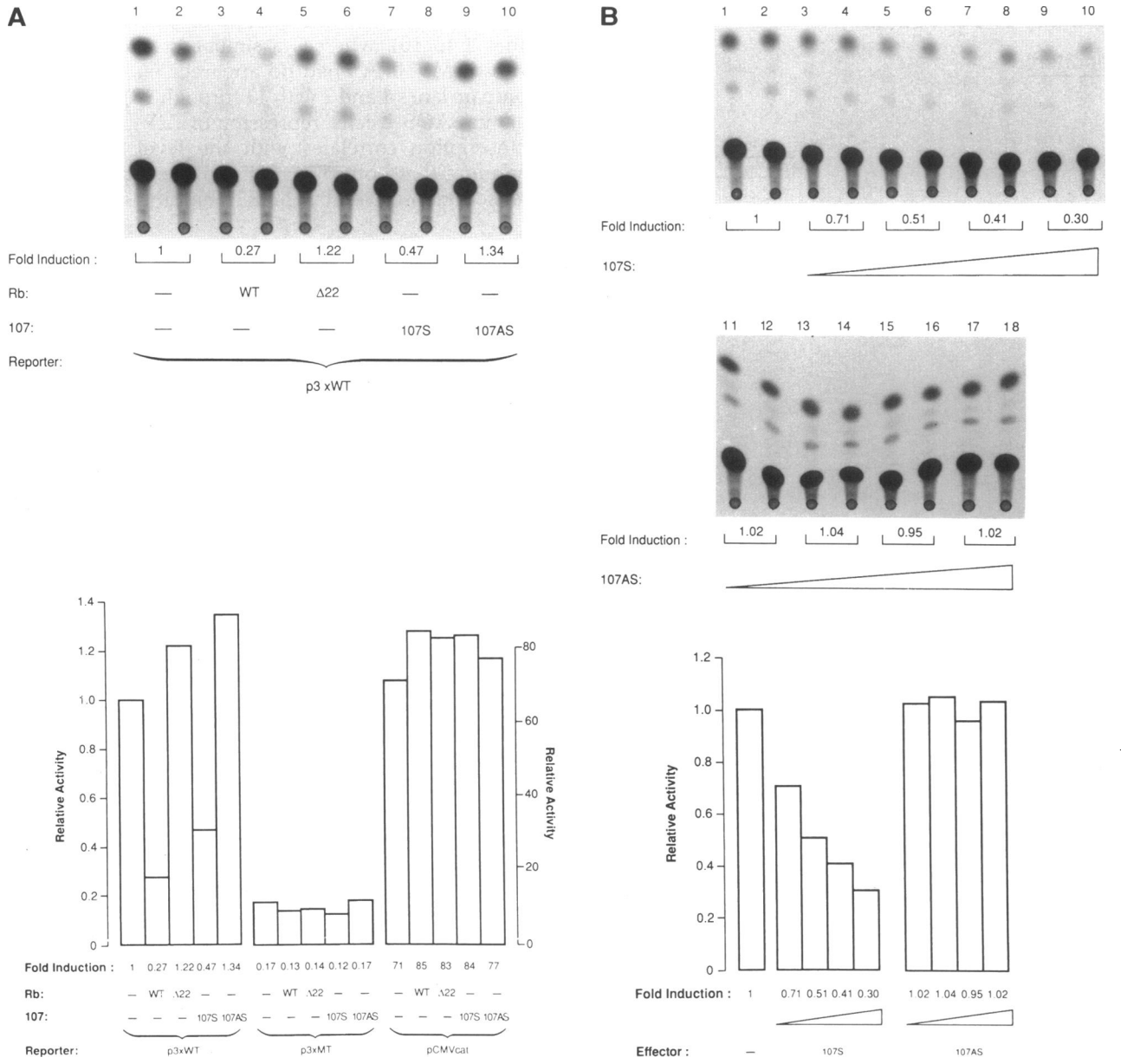

Figure 2. p107 represses E2F site-dependent transcription in SAOS-2 cells. (A) SAOS-2 cells (1.2×10^6) were transfected with either 5.0 μg of p3xWT or p3xMT or 0.5 μg of pCMVcat as indicated. Cotransfected effector plasmids were at 4.5 μg for pCMVHRb or pCMVHRbΔ22 or 5.0 μg for pCMV107S or pCMV107AS. For pCMVcat, either 0.8 μg of pCMVHRb or pCMVHRbΔ22 or 0.72 μg of pCMV107S or pCMV107AS was used. (B) The reporter construct p3xWT (5.0 μg) was cotransfected with increasing amounts (0.1, 1.0, 2.5, or 5.0 μg) of either pCMV107S (lanes 3–10) or pCMV107AS (lanes 11–18). All treatments were performed in duplicate and corrected for transfection efficiency with respect to pCMVβgal. All values are expressed relative to the activity of p3xWT alone, which was given an arbitrary value of 1.0, and are representative of at least three separate experiments. The scale on the right-hand side relates to the activity of pCMVcat.

and pCMVHRb because the level of repression of p3xWT correlated with the amount of expression vector transfected into cells (Figure 3B). As in SAOS-2 cells, pCMVHRb did not affect the activity of p3xMT or pCMVcat. Interestingly, p107 repressed transcription more efficiently in F9 PE cells than in SAOS-2 cells, whereas pRb repressed transcription more efficiently in SAOS-2 cells. The reasons for this remain to be explored.

We also determined the transfection efficiency of F9 PE cells using pCMV-βgal. F9 PE cells transfected marginally more efficiently than F9 EC cells (a typical example is shown in Figure 4B), but this difference was not sufficient to account for the distinct activities of pRb and p107 in F9 PE cells. pRb was also at similar levels in F9 PE as F9 EC cells (Figure 4, compare D and C). Furthermore, the amount of F9 EC and F9 PE cell ex-

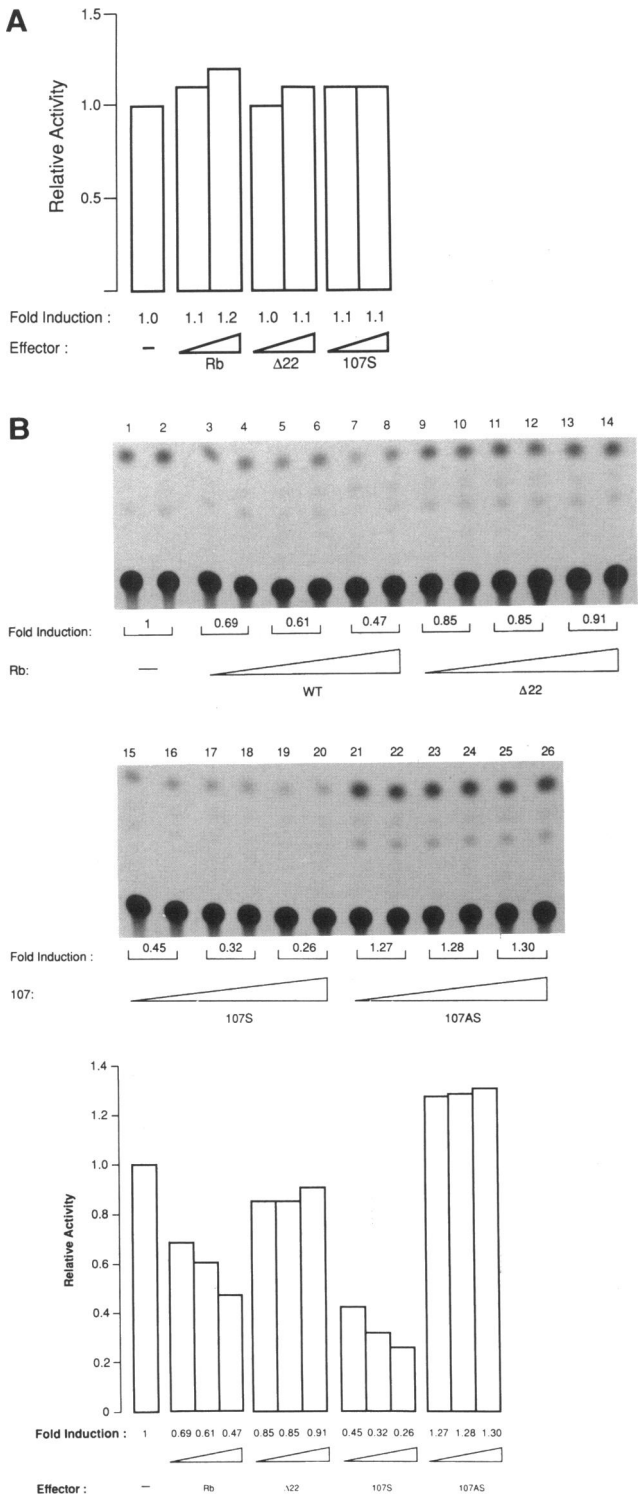

Figure 3. Repression of E2F site-dependent transcription by p107 and pRb is differentiation-regulated. (A) F9 EC (10^6) cells were co-transfected with p3xWT (5.0 μg) and two separate amounts of pCMVHRb or pCMVHRbΔ22 (4.5 and 13.5 μg) or pCMV107S (5.0 and 15 μg). All treatments were performed in duplicate and corrected for transfection efficiency with respect to pCMVβgal. (B) F9 PE (10^6) cells were transfected 3 d after differentiation had been induced (described in MATERIALS AND METHODS) with p3xWT (5.0 μg) and three separate amounts of pCMVHRb or pCMVHRbΔ22 (4.5, 9.0, and 13.5 μg) or pCMV107S or pCMV107AS (5.0, 10.0, and 15.0 μg). All treatments were performed in duplicates and were corrected for transfection efficiency with respect to pCMVβgal. Values are expressed with respect to the activity of p3xWT alone and are representative of at least three separate experiments.

tracts used in each assay was normalized with respect to the expression of pCMVβ-gal, a control for transfection efficiency (see MATERIALS AND METHODS). We conclude that both p107 and pRb can repress transcription in F9 cells but do so in a differentiation-dependent fashion.

Adenovirus E1a Overcomes p107-Mediated Transcriptional Repression

Adenovirus E1a binds p107 through regions that are necessary for the E1a protein to exert a range of biological effects, such as immortalization, transformation, and induction of DNA synthesis (Whyte *et al.*, 1989). We were therefore interested to determine the effect of the E1a 12S protein on the repressing activity of p107. Figure 5 confirms our previous observations, which indicated that E1a 12S (in pJ3Ω12S; Figure 1) can induce the transcriptional activity of p3xWT (Figure 5; compare tracks 1 and 2 with 3 and 4; three-fold induction), whereas E1a 12S had an insignificant effect on p3xMT (Zamanian and La Thangue, 1992). Coexpression of E1a with p107S overcame the repressing effect of p107 on p3xWT (Figure 5; compare tracks 13–16 with 5–8), indicating that E1a 12S prevents p107 from repressing E2F site-dependent transcription.

DISCUSSION

p107 Represses E2F Site-Dependent Transcription

Both p107 and pRb bind to DRTF1/E2F in a periodic fashion during cell-cycle progression (Mudryj *et al.*, 1991; Shirodkar *et al.*, 1992). It was predicted for pRb that this interaction would cause the repression of E2F site-dependent transcription for several reasons. First, pRb had an established role in negatively regulating cell-cycle progression (Goodrich *et al.*, 1991), and, consistent with this, *Rb* is often mutated in human tumor cells (Horowitz *et al.*, 1990; Hu *et al.*, 1990; Huang *et al.*, 1990). Second, E2F binding sites occur in the promoters of genes that encode proteins required for cellular proliferation (Blake and Azizkhan, 1989; Hiebert *et al.*, 1991; Pearson *et al.*, 1991; Dalton *et al.*, 1992), and thus repression of E2F site-dependent transcription could theoretically have influenced cellular proliferation. That pRb could repress E2F site-dependent transcription was established by several groups (Dalton, 1992; Hiebert *et al.*, 1992; Zamanian and La Thangue, 1992), thus

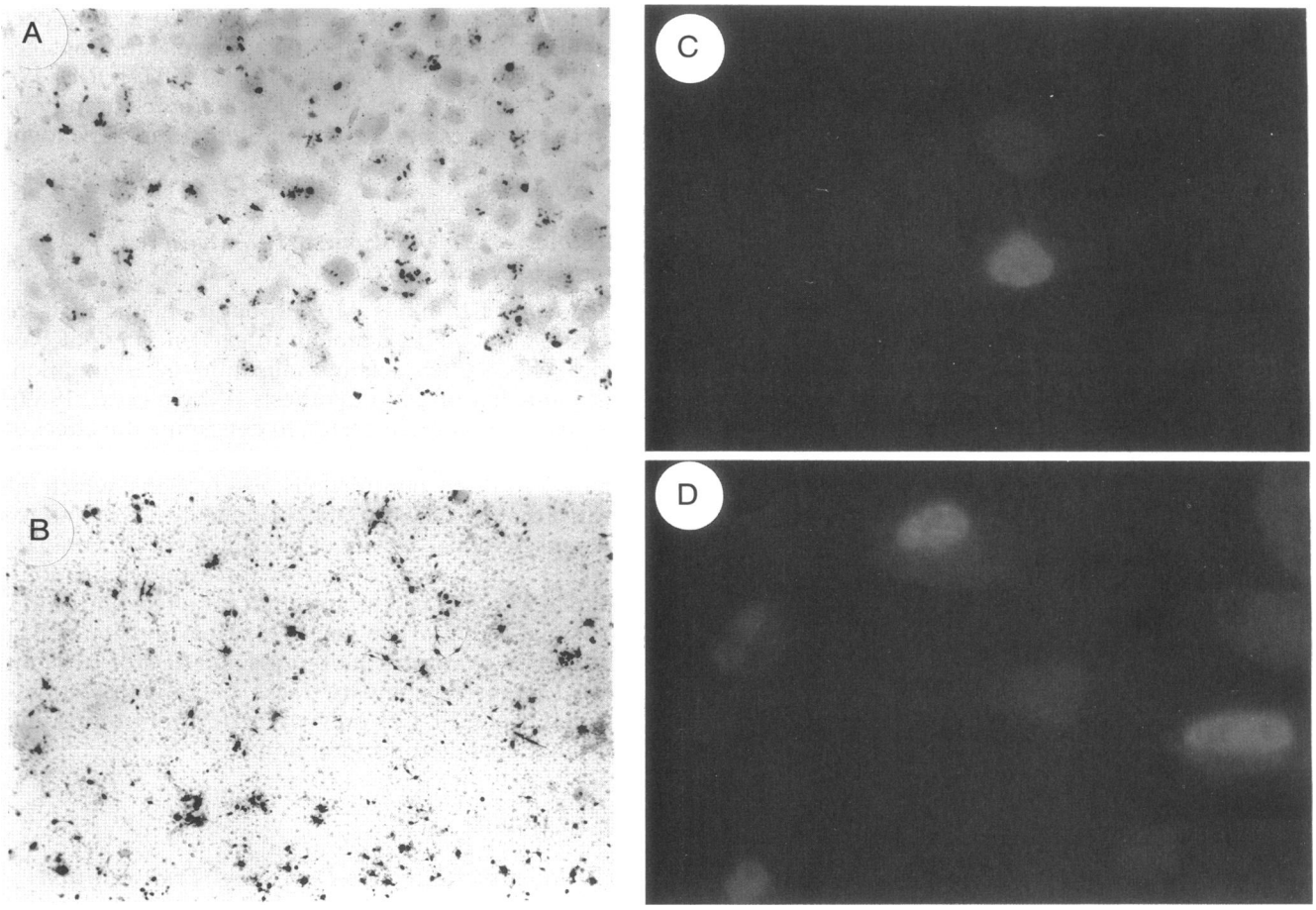

Figure 4. The human cytomegalovirus transcription control sequences are active in F9 EC cells and their differentiated derivatives. (A and B) F9 EC (10^6; A) and their differentiated derivatives, F9 PE (10^6; B), were transfected with 2.0 μg of pCMVβgal. Cells were fixed and stained for β-galactosidase activity as described in MATERIALS AND METHODS. (C and D) F9 EC (C) and their differentiated derivatives, F9 PE (D), were transfected with 20 μg of pCMVHRb. After 2 d, cells were stained with monoclonal antibody IF8, which recognizes human pRb (Bartek *et al.*, 1992). Immunofluorescence was performed as previously described (La Thangue, 1984). (C) and (D) show typical examples of IF8-stained nuclei in F9 EC and F9 PE cells, respectively. Magnification 630×.

providing a potential mechanistic explanation for the negative growth-regulating effect of pRb.

Although p107 is structurally related to pRb (Ewen *et al.*, 1991) and also binds to DRTF1/E2F (Cao *et al.*, 1992; Devoto *et al.*, 1992; Shirodkar *et al.*, 1992; Bandara *et al.*, 1993), the functional consequences of this interaction were less easy to predict. This is because several reports had shown that the p107 complex predominates during S phase (Mudryj *et al.*, 1991; Devoto *et al.*, 1992; Shirodkar *et al.*, 1992), which correlates with the transcriptional induction of some cellular promoters that contain E2F sites (Blake and Azizkhan, 1989; Hiebert *et al.*, 1991; Means *et al.*, 1992). Furthermore, the p107 gene has not so far been found to be mutated in tumor cells (Ewen *et al.*, 1991) and is therefore not known to possess the characteristics expected of a tumor suppressor gene and hence those of a negative growth regulator. The consequence of the interaction between p107 and DRTF1/E2F were thus difficult to predict.

The data presented in this study show that p107 behaves like pRb because it can repress the transcriptional activity of DRTF1/E2F. We believe, therefore, that p107 also may have a role in negatively regulating cell-cycle progression by modulating E2F site-dependent transcription. However, mutations in the p107 gene have not yet been identified, possibly because its activity is essential for cell survival. We believe that one potential explanation is that pRb regulates early cell-cycle progression and p107 late cell-cycle progression. Thus, once cells have entered the cycle (a decision that could involve pRb), p107 may function, for example, in monitoring the completion of S phase. It will be very interesting to determine the precise point at which p107 regulates cell-cycle progression.

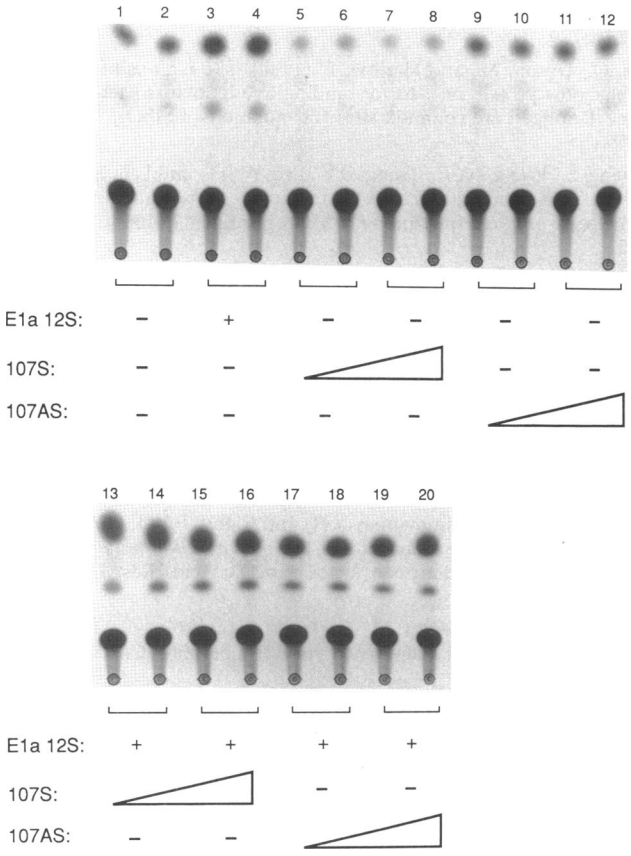

Figure 5. Adenovirus E1a overcomes p107-mediated transcriptional repression. SAOS-2 cells were cotransfected with 5.0 μg of p3xWT and the indicated effector plasmids. Where indicated, 2.0 μg of pJ3Ω12S was included. The plasmids pCMV107S and pCMV107AS were used at 5.0 and 15.0 μg. All treatments were performed in duplicate and corrected for transfection efficiency with respect to pCMVβgal. The values are representative of at least three different experiments.

Transcriptional Repression by pRb and p107 is Influenced by Differentiation

Although both pRb and p107 could repress E2F site-dependent transcription in F9 PE cells, neither protein affected transcription in F9 EC cells. We believe, therefore, that the biological activities of pRb and p107 are influenced by differentiation. We attempted to rule out trivial explanations for this effect by ensuring that the CMV sequences driving pRb and p107 were transcriptionally active and that pRb was synthesized in F9 EC cells. However, we would like to emphasize that we do not wish to conclude that pRb and p107 cannot regulate E2F site-dependent transcription in F9 EC cells (perhaps they could at higher concentrations than achieved in our assays) but rather that certain cells, such as F9 EC cells, are less sensitive to the regulatory effects of pRb and p107 than others.

It is interesting that a cellular E1a-like activity has been characterized previously in F9 EC cells because they allow E1a-dependent promoters in the adenovirus E1a deletion mutant d1312 to be transcribed (Imperiale *et al.*, 1984). Furthermore, this activity is regulated as F9 EC cells differentiate to F9 PE cells (Imperiale *et al.*, 1984; La Thangue and Rigby, 1987). An intriguing parallel between this cellular activity and the adenovirus E1a protein is with respect to the nature of DRTF1/E2F. Thus, adenovirus E1a dissociates complexed DRTF1/E2F by sequestering proteins, such as pRb and p107, releasing the transcriptionally active form of DRTF1/E2F, which is the form that also predominates in F9 EC cells (La Thangue *et al.*, 1990; Bandara and La Thangue, 1991; Partridge and La Thangue, 1991). DRTF1/E2F therefore exists in the same transcriptionally active state in both situations. We do not wish to imply that we believe there to be a cellular molecule that behaves like the adenovirus E1a protein. Rather, we suggest that the mechanisms that normally regulate the binding of pRb and p107 to DRTF1/E2F are sufficiently active in F9 EC cells that DRTF1/E2F is maintained in the transcriptionally active state, and in this respect the characteristics of F9 EC DRTF1/E2F resemble adenovirus E1a-modulated DRTF1/E2F. A potential explanation for the inability of pRb or p107 to repress DRTF1/E2F site-dependent transcription in F9 EC cells is that they could not override the cellular mechanisms that normally prevent pRb and p107 from binding to DRTF1/E2F. We believe that the F9 cell system will provide a very useful system for analyzing the mechanisms that control the biological activities of pRb and p107 and how these mechanisms are regulated during differentiation.

That the activity of pRb is influenced by the cell type also may be important for understanding the role of pRb during tumorigenesis. We suggest that this could be a possible explanation for why *Rb* is mutated in some, but not all, types of tumor cells (Horowitz *et al.*, 1990). This is because during the process of cellular transformation, mutations in *Rb* would be most likely to occur in cells where pRb is active in negative growth control and thus where its inactivation would provide a selective growth advantage.

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REFERENCES

Bandara, L.R., Adamczewski, J.P., Hunt, T., and La Thangue, N.B. (1991). Cyclin A and the retinoblastoma gene product complex with a common transcription factor. Nature *352*, 249–251.

Bandara, L.R., Adamczewski, J.P., Zamanian, M., Poon, R.Y.C., Hunt, T., and La Thangue, N.B. (1992). Cyclin A recruits $p33^{cdk2}$ to the cellular transcription factor DRTF1. In: Transcriptional Regulation in Cell Differentiation and Development, ed. P.W.J. Rigby, R. Krumlauf, and F. Grosveld. J. Cell. Sci. Supple. *16*.

Bandara, L.R., and La Thangue, N.B. (1991). Adenovirus E1a prevents the retinoblastoma gene product from complexing with a cellular transcription factor. Nature *351*, 494–497.

Bartek, J., Vojtesek, B., Grand, R.J.A., Gallimore, P.H., and Lane, D.P. (1992). Cellular localization and T antigen binding of the retinoblastoma protein. Oncogene *7*, 101–108.

Blake, M.C., and Azizkhan, J.C. (1989). Transcription factor E2F is required for efficient expression of hamster dihydrofolate reductase gene *in vitro* and *in vivo*. Mol. Cell. Biol. *9*, 4994–5002.

Cao, L., Faba, B., Dembski, M., Tsai, L.-H., Harlow, E., and Dyson, N. (1992). Independent binding of the retinoblastoma protein and p107 to the transcription factor E2F. Nature *355*, 176–179.

Chellappan, S.P., Hiebert, S., Mudryj, M., Horowitz, J.M., and Nevins, J.R. (1991). The E2F transcription factor is a cellular target for the Rb protein. Cell *65*, 1053–1061.

Chittenden, T., Livingston, D.M., and Kaelin, W.G., Jr. (1991). The T/E1A-binding domain of the retinoblastoma product can interact selectively with a sequence-specific DNA-binding protein. Cell *65*, 1073–1082.

Dalton, S. (1992). Cell cycle regulation of the human *cdc2* gene. EMBO J. *11*, 1797–1804.

Dannenberg, A.M., and Suga, M. (1981). Histochemical stains for macrophages in cell smears and tissue sections: β-galactosidase, acid phosphatase, non-specific esterase, succinic dehydrogenase, and cytochrome oxidase. In: Methods for Studying Mononuclear Phagocytes, ed. D.O. Adams, P.J. Edelson, and H.S. Koren, New York: Academic Press, 375–396.

Devoto, S.H., Mudryj, M., Pines, J., Hunter, T., and Nevins, J.R. (1992). A cyclin A-protein kinase complex possesses sequence-specific DNA binding activity:$p33^{cdk2}$ is a component of the E2F-cyclin A complex. Cell *68*, 167–176.

Ewen, M.E., Xing, Y., Lawrence, J.B., and Livingston, D.M. (1991). Molecular cloning, chromosomal mapping, and expression of the cDNA for p107, a retinoblastoma gene product-related protein. Cell *66*, 1155–1164.

Goodrich, D.W., Wang, N.P., Qian, Y.-H., Lee, E.Y.-H., and Lee, W.-H. (1991). The retinoblastoma gene product regulates progression through the G1 phase of the cell cycle. Cell *67*, 293–302.

Hiebert, S.W., Blake, M., Azizkhan, J., and Nevins, J.R. (1991). Role of E2F transcription factor in E1A-mediated *trans* activation of cellular genes. J. Virol. *65*, 3547–3552.

Hiebert, S.W., Chellappan, S.P., Horowitz, J.M., and Nevins, J.R. (1992). The interaction of RB with E2F coincides with an inhibition of the transcriptional activity of E2F. Genes Dev. *6*, 177–185.

Hiebert, S.W., Lipp, M., and Nevins, J.R. (1989). E1A-dependent transactivation of the human MYC promoter is mediated by the E2F factor. Proc. Natl. Acad. Sci. USA *86*, 3594–3598.

Hogan, B.L.M., Taylor, A., and Adamson, E. (1981). Cell interactions modulate embryonal carcinoma cell differentiation into parietal or visceral endoderm. Nature *291*, 235–237.

Horowitz, J.M., Park, S.-H., Bogenmann, E., Cheng, J.-C., Yandell, D.W., Kaye, F.J., Minna, J.D., Dryja, T.P., and Weinberg, R.A. (1990). Frequent inactivation of the retinoblastoma anti-oncogene is restricted to a subset of human tumour cells. Proc. Natl. Acad. Sci. USA *87*, 2775–2779.

Hu, Q., Dyson, N., and Harlow, E. (1990). The regions of the retinoblastoma protein needed for binding to adenovirus E1A or SV40 large T antigen are common sites for mutations. EMBO J. *9*, 1147–1155.

Huang, S., Wang, N.-P., Tseng, B.Y., Lee, W.-H., and Lee, E.H.-H.P. (1990). Two distinct and frequently mutated regions of retinoblastoma protein are required for binding to SV40 T antigen. EMBO J. *9*, 1815–1822.

Imperiale, M.J., Kao, H.-T., Feldman, L.T., Nevins, J.R., and Strickland, S. (1984). Common control of the heat shock gene and early adenovirus genes: Evidence for a cellular E1A-like activity. Mol. Cell. Biol. *4*, 867–874.

La Thangue, N.B. (1984). A major heat-shock protein defined by a monoclonal antibody. EMBO J. *3*, 1871–1879.

La Thangue, N.B., and Rigby, P.W.J. (1987). An adenovirus E1A-like transcription factor is regulated during the differentiation of murine embryonal carcinoma stem cells. Cell *49*, 507–513.

La Thangue, B.B., Thimmappaya, B., and Rigby, P.W.J. (1990). The embryonal carcinoma stem cell E1a-like activity involves a differentiation-regulated transcription factor. Nucleic Acids Res. *18*, 2929–2938.

Lillie, J.W., Loewenstein, P.M., Green, M.R., and Green, M. (1987). Functional domains of adenovirus type 5 E1a proteins. Cell *50*, 1091–1100.

Means, A.L., Slansky, J.E., McMahon, S.L., Knuth, M.W., and Farnham, P.J. (1992). The HIP1 binding site is required for growth regulation of the dihydrofolate reductase gene promoter. Mol. Cell. Biol. *12*, 1054–1063.

Moran, E., and Mathews, M.B. (1987). Multiple functional domains in the adenovirus E1a gene. Cell *48*, 177–178.

Morris, J.D.H., Crook, T., Bandara, L.R., Davies, R., La Thangue, N.B., and Vousden, K.H. (1993). HPV16 E7 regulates E2F and contributes to mitogenic signalling. Oncogene (*in press*).

Mudryj, M., Devoto, S.H., Hiebert, S., Hunter, T., Pines, J., and Nevins, J.R. (1991). Cell cycle regulation of the E2F transcription factor involves an interaction with cyclin A. Cell *65*, 1243–1253.

Partridge, J.F., and La Thangue, N.B. (1991). A developmentally regulated and tissue-dependent transcription factor complexes with the retinoblastoma gene product. EMBO J. *10*, 3819–3827.

Pearson, B.E., Nasheuer, H.-P., and Wang, T.S.-F. (1991). Human DNA polymerase alpha gene: Sequences controlling expression in cycling and serum-stimulated cells. Mol. Cell. Biol. *11*, 2081–2095.

Shirodkar, S., Ewen, M., DeCaprio, J.A., Morgan, J., Livingston, D.M., and Chittenden, T. (1992). The transcription factor E2F interacts with the retinoblastoma product and a p107-cyclin A complex in a cell cycle-regulated manner. Cell *68*, 157–168.

Tassios, P.T., and La Thangue, N.B. (1990). A multiplicity of differentiation-regulated ATF site-binding activities in embryonal carcinoma cells with distinct sequence and promoter specificities. New Biol. *12*, 1123–1134.

Whyte, P., Williamson, N.M., and Harlow, E. (1989). Cellular targets for transformation by the adenovirus E1A proteins. Cell *56*, 67–76.

Zamanian, M., and La Thangue, N.B. (1992). Adenovirus E1a prevents the retinoblastoma gene product from repressing the activity of a cellular transcription factor. EMBO J. *11*, 2603–2610.