The retinoblastoma protein (pRB) regulates cell cycle progression by interacting with a number of cellular proteins. Binding to E2F transcription factors inhibits activation of S-phase transcriptional target genes, such as cyclin E, B-myb, and PCNA, among others (20, 47). Recruitment of LXCXE-containing chromatin-remodeling proteins, such as histone methyltransferases (Suv39H1 and -2) and histone deacetylases (HDAC1 and -2) to these promoters by pRB serves to actively control the accessibility of target gene promoters and further regulates transcription (6, 27, 30, 31, 36). Recently, it has also been shown that pRB can regulate cyclin/cdk activity directly by using APCcdh1 to antagonize Skp2’s ability to degrade p27 (5, 22). Under normal proliferative conditions, mitogen stimulation leads to inactivation of pRB through G1, cyclin/cdk complexes that phosphorylate pRB and disrupt its ability to bind to its regulatory targets (13).

In a remarkably similar fashion, advancement of the cell cycle into S phase can arise from expression of small DNA tumor virus proteins, such as adenovirus E1A (25, 43). E1A drives transcription of early viral genes and through interaction with pRB and other proteins induces S-phase entry (34). A key activity in E1A’s ability to stimulate transcription and viral propagation is the disruption of E2F transcription factor regulation by the retinoblastoma family of proteins. Collectively, E2Fs were originally described as cellular transcription factors required for the transcriptional activation of the adenovirus E2 promoter (26). Currently, there are eight distinct E2F family proteins, and each is known to be capable of binding to the E2F consensus DNA sequence element (23). However, not all of these are potent activators of transcription or interact with RB family proteins. Since E2Fs 1 to 5 interact with pRB family proteins (46), the disruption of these interactions is of particular interest for understanding how E1A functions in directing viral gene transcription.

Two conserved regions (CR) within E1A have been shown to be necessary for disruption of pRB-E2F transcription factor complexes (39). E1A CR1 and CR2 show high protein sequence similarity among numerous E1A serotypes (1), as well as to regions in viral oncogenes from other DNA tumor viruses (18, 38). The LXCXE motif within CR2 of E1A forms a strong interaction with the pRB pocket domain (16). Once tethered to pRB, the CR1 region of E1A mediates disruption of E2F binding (16). CR1 can make a relatively weak interaction with pRB on its own because it resembles the portion of E2Fs that contact pocket proteins (14, 29). This suggests that E2Fs are ultimately removed from pRB by competition with CR1 (16). At present there is little indication why E1A uses a multistep mechanism rather than direct competition to disrupt E2F regulation.

In addition to regulating proliferation, E2F1 has distinguished itself from other E2Fs by its unique roles in apoptosis and DNA repair (23). Endogenous E2F1 is activated by DNA damage signaling and can induce apoptosis (28, 37, 44). Thymocytes from E2F1 knockout mice are resistant to apoptosis induced by DNA damage and during T-cell development (17, 28). In addition, these mice frequently succumb to lymphomas, suggesting E2F1 may use this activity to function as a tumor suppressor gene (50). Conversely, endogenous E2F1 has also been demonstrated to regulate expression of genes necessary for DNA repair and maintenance of cell viability (40). In this regard E2F1−/− mice are more prone to apoptosis in the epidermis following DNA damage, and this correlates with an inability to engage DNA repair mechanisms (4, 49). This background emphasizes that E2F1 has additional functions that...
modulate cell viability to cause cell death or to promote survival, and for this reason it is different from other E2F family proteins. How the cellular context controls which E2F1 activity is activated by DNA damage or other stimuli remains speculative (23). Following infection of a host cell, E1A is the first adenoviral protein expressed. The need to maintain cell viability and induce S-phase entry by adenoviruses at this stage of infection is underscored by the observation that other early viral proteins are potent apoptotic inhibitors (3). Given the necessity to induce cell cycle advancement while maintaining viability early after viral infection, it seems counterintuitive that E1A would deregulate E2F1 along with the other E2Fs. It seems more logical that E1A would want to manipulate E2F1 and its specialized roles to help maintain cell viability.

It has previously been demonstrated that pRB contains two independent binding sites for E2F transcription factors (7, 10, 23). One type of pRB-E2F interaction is termed the general interaction, because it is unspecific for its E2F binding partner and appears to function mainly to regulate cell cycle advancement (10). The second, specific, E2F binding site on pRB is used exclusively by E2F1, and this interaction has previously been shown to provide negative regulation of apoptosis while having almost no impact on proliferative control (24). Based on this division of function between different E2F binding configurations with pRB, we wondered if E1A was also selective in its ability to regulate E2F1. In this report we demonstrate that E1A is unable to disrupt E2F1 binding to pRB. In transfection experiments, E1A disrupted only the proliferative control, or general, interaction between pRB and E2F1. During a productive adenoviral infection where viruses infect quiescent cells, induce advancement of the cell cycle, and replicate, endogenous E2F1-pRB complexes are selectively retained. Furthermore, our analysis reveals that E2F1 functions to maintain viability following expression of E1A. These data strongly argue that E1A has developed a mechanism of selectively disrupting proliferative control mechanisms by pRB while leaving a specialized form of E2F1 regulation intact to maintain cell viability.

MATERIALS AND METHODS

Cell culture. RB-1-deficient C33A cervical carcinoma cells, IMR90 human diploid fibroblasts, and 293 human embryonic kidney cells were obtained from the American Type Culture Collection. Mouse embryonic fibroblasts (MEFs) were generated from the appropriate genotypes of mice using previously published methods (21). All cells were cultured in Dulbecco’s modified Eagle’s medium supplemented with penicillin (100 U/ml), l-glutamine (200 mM), streptomycin (10 μg/ml), and 10% fetal bovine serum. Cells were maintained in a humidified chamber at 37°C with 5% CO2.

Plasmids and recombinant proteins. CMV-β-Gal, CMV-HA-DP1, CMV-HA-E2F1, CMV-HA-E2F4, CMV-RB, and CMV-RB38EG-FG have all been previously described (10). CMV-RB38EG-FS was constructed similarly to other pRB mutants and has been described by Julian et al. (24). Plasmid pEGFP-E1A12S was obtained from S. Lowe (Cold Spring Harbor Laboratory) and XhoI and ligating into a pGEX-based GST-E1A12S plasmid as above. E1A12S.Y47H was constructed by digesting pEGFP-E1A12S.Y47H with EcoRI and its specialized roles to help maintain cell viability.

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RESULTS

GST-E1A is unable to disrupt pRB-E2F1 complexes. Based on evidence that pRB uses two distinct interaction mechanisms to contact E2F transcription factors (7, 10, 24), we sought to investigate how E1A affects these different interaction types. To this end we devised an in vitro competition assay to study pRB-E2F interactions. Briefly, RB-1-deficient C33A cells were transfected with pRB, HA-E2F1, and HA-DP1 or pRB, HA-E2F4, and HA-DP1. Extracts were analyzed by Western blotting, and input amounts were normalized to the expression level of pRB (Fig. 1A). Extracts were supplemented with a range of GST-E1A12S protein concentrations or GST only, as a control. E2F transcription factors were immunoprecipitated with anti-HA antibodies, and coprecipitated pRB was detected by Western blotting (Fig. 1B). Addition of GST-E1A12S resulted in a clear disruption of E2F4-pRB-containing complexes (Fig. 1B, top panel); however, E2F1-pRB interactions were insensitive to GST-E1A12S at all concentrations tested (Fig. 1B, bottom panel). This suggests that there is an inherent resistance of E2F1-pRB-containing complexes to GST-E1A12S-mediated disruption that is orders of magnitude stronger than E2F4. This is even more remarkable considering that the input level of pRB was similar in these experiments. Each lane represents 2.5% of the total input used in the IP experiments shown in panels B to D. Asterisks mark irrelevant bands that cross-react with HA antibodies. A 10-fold dilution of GST-E1A12S (B) or GST-E1A13S (C) from 10 μg to 10 ng was added to extracts expressing pRB and either HA-E2F4 or pRB and HA-E2F1. The quantity of pRB captured with E2Fs by IP was analyzed by Western blotting. (D) Tenfold dilutions of GST-E1A12S.Y47H or GST-E1A12S.d1107 were used similarly in extracts containing HA-E2F4 and pRB. The negative control (-CTRL) lane was generated by anti-HA IPs of cell extracts expressing pRB but not HA-E2Fs.

This demonstrates that both the 12S and 13S forms of E1A can dissociate pRB-E2F4 complexes with as little as 100 ng of GST-E1A protein, while pRB-E2F1 interactions are resistant, even at GST-E1A protein levels that are 100 times higher. To verify that E2F4-pRB complexes were being dissociated by functional GST-E1A, we utilized GST-tagged E1A proteins with mutations in the conserved regions to test if our competition assay relies on the same regions of E1A as previously described (16, 39). The LXCXE-containing region (CR2) normally mediates the initial contact between E1A and pRB, while conserved region 1 (CR1) contains an E1A-pRB interaction region that competes with E2Fs for binding to pRB. GST-E1A12S containing a Y47H amino acid substitution within CR1 has a diminished ability to compete with E2F4 for binding to pRB (Fig. 1D, top panel). GST-E1A12S.d1107 contains a deletion of residues 111 to 123 within CR2 that interrupts the LXCXE motif and causes a loss of pRB binding. This CR2 mutation abrogates E1A’s ability to disrupt E2F4-pRB complexes (Fig. 1D, bottom panel). These observations validate the experiments outlined in Fig. 1A to C, which indicate that GST-E1A12S is a functional E1A protein capable of E2F4-pRB dissociation activity but lacking the ability to disrupt E2F1-pRB.
E1A uses a separate mechanism to regulate pRB-E2F1 interactions. We recently discovered that pRB contains a second E2F interaction site that uniquely regulates E2F1 (10, 24). This interaction was mapped to distinct regions of both E2F1 and pRB, involving the C-terminal region of the large pocket of pRB and a small portion of the E2F1 marked box region (24, 41). Thus, E2Fs can bind to pRB by a common mechanism at a location that we have termed the “general” site (or G, for brevity). The interaction mechanism that is unique to E2F1 occurs in a region referred to as the “specific” site (or S), and these are depicted in a cartoon in Fig. 2A. Since E2F1 can interchangeably use these two interaction mechanisms, we have generated mutants defective for each type of interaction alone to study their functions in isolation (10, 24). These mutations allowed us to examine both interaction sites independently, because a combination allele carrying both types of mutations in the same molecule eliminates all E2F1 binding (24).

To examine if one of the pRB-E2F1 interactions was responsible for the resistance to GST-E1A-mediated disruption, as depicted in Fig. 1, we sought to investigate how E1A binds to pRB and ultimately competes with E2Fs. C33A cells were transiently transfected with plasmids encoding pRB, pRBΔG, or pRBΔS, and expression levels of these proteins are shown in Fig. 2B. The ability of these expressed proteins to bind to E1A was measured by GST–pull-down assays. When GST-E1A12S was mixed with extracts expressing pRBΔG, the E1A-pRB interaction was lost in the 100-ng GST-E1A12S pull-down sample (Fig. 2C). This suggests that E1A binds in part through the general E2F binding site and the pRBΔG mutant reduces the affinity of this interaction. To investigate pRB binding further, we used CR1 and CR2 mutants to examine the contribution of each region of E1A in isolation. The use of GST-E1A12S.Y47H demonstrates that the affinity of E1A CR2 for each of the wild-type, pRBΔG, and pRBΔS proteins is the same and thus the LXCXE interaction with pRB is unaffected by either pRB mutation (Fig. 2D). In contrast, when GST-E1A12S.dl1107 was mixed with extracts expressing pRB and pRB-E2F binding mutants, a complete loss of E1A-pRB association was detected for pRBΔG (Fig. 2E). This finding demonstrates that the CR1 region is essential for contacting the general E2F binding site. Thus, CR1 competition for E2F binding is through direct contact with the same region of pRB. This further suggests that E2F1, but not E2F4, may evade this disruption by using a separate binding site on pRB.

To test if E2F1 resistance to E1A displacement is dependent on the specific binding site, cells were transfected with plasmids encoding HA-E2F1 and HA-DP1 and either pRBΔG or pRBΔS. Input levels of pRB and HA-E2F1/HA-DP1 used in these assays are shown in Fig. 3A. Using the same competition assay approach as in Fig. 1, we analyzed the ability of GST-E1A12S to disrupt HA-E2F1/HA-DP1 binding to either pRBΔG or pRBΔS. As shown in Fig. 3B, neither interaction was dis-

![FIG. 2. E1A CR1 contacts the general E2F binding site on pRB. (A) Schematic diagram illustrating the two different E2F-pRB interaction types. The general (G) E2F binding site on pRB interacts with E2Fs without preference. E2F1 can bind to pRB through either the G or the E2F1 S (specific) site. Mutant forms of pRB that lack either the general or specific interaction site allow the other E2F interaction type to be studied in isolation. (B) C33A whole-cell extracts from cells expressing the indicated RB constructs were analyzed for relative pRB expression. Each lane represents 2% of the input used in each pull-down experiment. (C) Recombinant GST-E1A12S was added to transfected cell extracts in a 10-fold titration range from 10 μg to 100 ng. Bound wild-type or mutant pRB was collected on glutathione-Sepharose beads and detected by Western blotting. Similar analyses of CR1 (Y47H) and CR2 (dl1107) mutants in GST-E1A12S were carried out (D and E).]
E2F/HA-DP1 was immunoprecipitated. When E1A12S-extracted extracts were added to E2F/pRB-containing extracts, and HA-translational modifications (Fig. 3C). E1A-containing nuclear were harvested to obtain E1A carrying the appropriate post-translational modifications (Fig. 1). E1A12S was expressed in mammalian cells capable of competing complexes containing E2F4 and pRB, indicating that E1A used in these experiments is active. We also tested the ability of this endogenous form of E1A to compete for binding with E2F1 at each individual binding site on pRB. As shown in Fig. 3E, neither RBΔG-E2F1 nor RBΔS-E2F1 complexes could be disrupted by E1A12S expressed in mammalian cells. These findings indicate that there is a fundamental difference between E2F1 and E2F4 binding to pRB, and it is manifested in resistance to E1A-mediated disruption, even when these E2Fs bind to the same region of pRB.

To investigate E1A regulation of E2F1-pRB complexes further, we tested competition in transfection assays to see if E1A could break this interaction in vivo. C33A cells were cotransfected with constructs for the expression of HA-E2F1 or HA-E2F4, pRB, and E1A12S where indicated. HA-tagged E2F-DP1 complexes were immunoprecipitated, and the persistence of pRB in these E2F complexes was detected by Western blotting. As shown in Fig. 4A, wild-type pRB associates with E2F1 in the presence of E1A to the same extent as when E1A is absent, indicating that E1A is unable to quantitatively disrupt E2F1-pRB complexes. However, when E1A is coexpressed with E2F4, disruption of E2F4-pRB complexes is observed. To explore the two types of E2F1 interactions in isolation, RBΔG- and RBΔS E2F-binding mutants were coexpressed in combination with HA-E2F1/HA-DP1 either in the absence or presence of E1A (Fig. 4B and C). HA-E2F1 complexes captured by IP coprecipitate with pRBΔG equally, indicating that interactions through the specific E2F1 regulatory site are not disrupted by E1A. However, when E1A is coexpressed with complexes consisting of pRBΔS and HA-E2F1, a clear competition with E2F1 binding to pRB is detected (Fig. 4C). This indicates that although resistant to E1A-mediated disruption, pRB-E2F1 complexes can be dissociated by E1A from the general, cell cycle regulatory binding site, but the specific E2F1 only configuration remains undisturbed. The fact that the pRB-E2F1 general interaction is only competed by E1A in an intact cell suggests that there are mechanistic distinctions between disruption of this interaction and disruption of E2F4-pRB, and these possibilities will be elaborated on in the Discussion section, below.

Analysis of in vivo E2F-pRB interactions reveals the maintenance of endogenous pRB-E2F1 complexes in the presence of E1A. Most analyses of E1A-mediated disruption of pRB-E2F interactions have used heterogeneous pocket protein-E2F complexes found in cell lysates (2, 8, 9, 16, 39). Because most of these reports predate the molecular cloning of individual E2F family members, we wondered if E2F1 is retained in endogenous complexes with pRB despite the presence of E1A.

We investigated whether E2F1 is retained in complexes with pRB during a productive adenoviral infection. We used primary human IMR90 cells that were confluent, and therefore growth arrested, to determine if this interaction is preserved throughout viral replication. As shown in Fig. 5A, E1A expression is detectable across the time course of infection and peaks at 24 h, which is indicative of the infection, replication, and lysis that take place over a complete infectious cycle. Using

![Diagram](http://jvi.asm.org/)
nuclear extracts from these cells, we immunoprecipitated pRB and performed Western blotting to determine the relative abundance of E2F proteins that associate with pRB. These experiments revealed that E2F1 remains associated with pRB when E2F3’s interaction is clearly disrupted. To examine the selective retention of E2F1-pRB complexes in more detail, we devised a quantitative immunoprecipitation assay to detect relative pRB-E2F abundance for all E2F complexes. HEK293 cells stably express E1A, making them an ideal source of material for these experiments. Nuclear extracts were subjected to three rounds of immunoprecipitation using antiserum directed against the C terminus of pRB. The pooled IP fractions contain nearly all pRB from the nuclear extract, and comparison with the unbound supernatant fraction allows the estimation of the percentage of the protein immunoprecipitated. Figure 5B illustrates that E2Fs 2 to 4 remained largely unassociated with pRB in the presence of E1A, but the majority of nuclear E2F1 was bound to pRB. Quantification of pRB binding to E2Fs in Fig. 5B illustrates that E2Fs 2 to 4 remained largely unassociated with pRB in the presence of E1A, but the majority of nuclear E2F1 was bound to pRB. Quantification of pRB binding to E2Fs in Fig. 5B illustrates that E2Fs 2 to 4 remained largely unassociated with pRB in the presence of E1A, but the majority of nuclear E2F1 was bound to pRB. Quantification of pRB binding to E2Fs in Fig. 5B illustrates that E2Fs 2 to 4 remained largely unassociated with pRB in the presence of E1A, but the majority of nuclear E2F1 was bound to pRB. Quantification of pRB binding to E2Fs in Fig. 5B illustrates that E2Fs 2 to 4 remained largely unassociated with pRB in the presence of E1A, but the majority of nuclear E2F1 was bound to pRB.
E2F1 functions to promote viability following E1A expression. Our experiments reveal the existence of a pRB-E2F1 complex that is selectively retained during adenoviral infection. Furthermore, they indicate that E2F1, pRB, and E1A form a stable complex, with E2F1 most likely bound to pRB through its specific interaction. To investigate the physiological purpose for this complex, we devised assays to test the functions for pRB and E2F1 following ectopic expression of E1A, a situation that mimics early adenoviral infection. E1A expression alone is capable of inducing cell death in this primary cell culture system under conditions of serum deprivation or DNA damage (33, 42). Likewise, E2F1 expression is induced by DNA damage, suggesting that it offers the most physiologically relevant condition under which to investigate how activation of E2F1 function can modulate viability or induce apoptosis early in adenoviral infection (4, 28, 37, 44, 49). In these experiments we used Rb<sup>+/−</sup> MEFs, which express a mutant form of pRB that contains mutations in its LXCXE binding cleft to prevent binding to CR2 in E1A (12, 21), and E2f1<sup>/−</sup> cells as well as wild-type controls. Expression of E1A in these cells was facilitated by retroviral transduction and drug selection (42).

The Western blots in Fig. 6A show the uniform expression of E1A and pRB in the three genotypes of MEFs. In addition, E1A can coprecipitate pRB in wild-type and E2f1<sup>/−</sup> cells, but
not \( Rb^{ΔL/L} \) MEFs. In this way our assay system allows us to determine the effect of blocking E1A access to all pRB-E2F complexes, as well as allowing it to interact with pRB (and disrupt E2F2, -3, and -4 interactions) in the absence of E2F1. We assayed the effect of expressing E1A on cell viability in the different genotypes of MEFs to determine if pRB-E2F regulation plays a role in maintaining viability. In Fig. 6C, E1A or vector control cells were treated with etoposide to induce double-stranded DNA breaks that would activate E2F1. This analysis revealed that preventing E1A from contacting pRB provides protection from cell death. Interestingly, allowing E1A to disrupt other pRB-E2F complexes in the absence of E2F1 renders cells more sensitive to DNA damage-induced death. In a similar experiment, cells were also subjected to serum deprivation (Fig. 6B). Again, the \( Rb^{ΔL/L} \) mutation protected cells, while the lack of E2F1 conferred an increased sensitivity to cell death.

Previous work by Samuelson et al. shows that E1A CR1 and CR2 are necessary for DNA damage-associated cytotoxicity (42); however, this activity of E1A only disrupts E2F2, -3, and -4 binding to pRB, suggesting that cell death is caused by a conflict in proliferative signals from E1A and arrest signals from DNA damage in a manner that is similar to what has been suggested for serum starvation combined with E1A expression (33). Our work indicates that E2F1, likely in complex with E1A and pRB, contributes to the maintenance of cell viability in response to DNA damage and E1A. This effect on cell viability suggests why it is advantageous for E1A to regulate E2F1 differently than other E2Fs immediately after viral infection.

**DISCUSSION**

This report describes a mechanism to explain how E1A disrupts the binding of E2Fs to one site of pRB while avoiding disruption of the E2F1 interaction at a second contact site on pRB. The selective activity of E1A is illustrated in Fig. 7, in which we show that an interaction between pRB and E2F4 is disrupted by E1A through the binding of CR1 to pRB to block E2F binding at the “general” interaction site (Fig. 7A). In contrast, when E2F1 is bound to pRB through the E2F1 “specific” site, E1A is unable to disrupt this protein complex (Fig. 7B). Furthermore, we demonstrate for the first time that E2F1 functions to maintain cell viability in response to external signals in the presence of E1A.
The in vitro competition assays used in this report reveal a striking difference in sensitivity between E2F4 and E2F1 in their dissociation from pRB by recombinant E1A. Since pRB preferentially regulates activator E2Fs over repressors like E2F4 (46), one possible interpretation is that this assay merely reflects differences in binding affinity for pRB. We do not think differences in affinity can explain this result. As shown in Fig. 4A, E2F1 and E2F4 immunoprecipitations of pRB in the absence of E1A result in only slightly less pRB in E2F4 IPs compared to E2F1. Thus, the dramatically more resistant behavior of pRB-E2F1 complexes to E1A is unlikely to be explained by a small difference in pRB-E2F2 affinity.

Our studies manipulated the region of pRB where E2F1 could bind and then tested the sensitivity to E1A-mediated disruption. These assays showed an inability of E1A to disrupt E2F1 from binding to either the specific or general regulatory sites in vitro but an ability to disrupt the general interaction in vivo (Fig. 3 and 4). It is possible that this difference is due to the fact that recombinant E1A12S doesn’t have sufficient opportunity to disrupt E2F1-pRBΔS, whereas expression of E1A concurrently with pRB and E2F1 in vivo may prevent these complexes from forming. It is also possible that heat shock proteins coexpressed with E1A in vivo participate in liberating E2F1 from the general site in an intact cell but are functionless in an in vitro assay (35, 48). Regardless of the explanation for this discrepancy, the experimental differences between E1A disruption of E2F4 and E2F1 at the same site on pRB emphasize that E1A recognizes E2F1 as unique among E2Fs.

The analysis of pRB-E2F complexes during adenoviral infection and in HEK293 cells reveals that endogenous pRB readily interacts with E2F1 in the presence of E1A, but not with other E2Fs (Fig. 5). It seems surprising that this specificity has been overlooked until now. However, previous experiments to investigate the mechanism used by E1A to disrupt pRB-E2F complexes examined disruption of a mixed population of E2Fs and couldn’t have made this distinction (2, 8, 9, 16, 39). The most obvious implication of the preferential maintenance of pRB-E2F1 interactions in the presence of E1A is that E2F1 functions differently than other E2F family members. Since E2F1 has previously been shown to preserve viability through the induction of DNA repair (4, 49), perhaps this unique interaction is not surprising. Our experiments reveal that E2F1 functions to maintain cell viability in response to growth factor deprivation, or following DNA damage, when E1A is present. We think this is highly provocative, because these experiments mimic the susceptibility of newly infected cells to exogenous signals that could kill the host cell and prevent viral replication. The mechanism by which E2F1 has this viability-promoting effect is presently unclear; however, we envision two general possibilities. One is that the stable E2F1-pRB-E1A complex actively provides negative regulation of apoptosis through further protein-protein interactions that modify cell viability signaling pathways, or regulation of apoptotic target gene expression. For example, this complex could occupy promoters that accommodate the pRB-E2F1 “specific” configuration and along with E1A regulate transcription of genes that affect cell viability. Alternatively, it is possible that the functional connection between E1A and loss of E2F1 in cell death is a relatively indirect effect of separate regulatory mechanisms. In this interpretation, the E1A and E2F1 functional connection could be mediated by something as simple as competition between these two transcription factors for a common cofactor like p300.

One implication of our work is that the differential effects that E1A exerts on the general and specific pRB-E2F complexes is a very precise means of selectively inducing cell cycle advancement in infection and transformation while manipulating E2F1 to maintain cell viability. This extends our understanding of the mechanism by which viral proteins such as E1A deregulate cellular growth control and raises the possibility that there is a class of E2F target genes, controlled by E2F1, which retain normal regulation despite the effects of E1A on pRB.

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