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E2F: A Link Between the Rb Tumor Suppressor Protein and Viral Oncoproteins

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The cellular transcription factor E2F, previously identified as a component of early adenovirus transcription, has now been shown to be important in cell proliferation control. E2F appears to be a functional target for the action of the tumor suppressor protein Rb that is encoded by the retinoblastoma susceptibility gene. The disruption of this E2F-Rb interaction, as well as a complex involving E2F in association with the cell cycle–regulated cyclin A–cdk2 kinase complex, may be a common mechanism of action for the oncoproteins encoded by the DNA tumor viruses.

Considerable attention has recently focused on the tumor suppressor genes and their role in the regulation of cell proliferation (*1*). One intensely studied example is the retinoblastoma susceptibility gene (*RB1*). Loss of *RB1* function is associated with the loss of cellular proliferative control, and the introduction of a wild-type *RB1* gene into cells that lack *RB1* can suppress cell growth and tumorigenicity (*2*, *3*). Moreover, injection of the *RB1* gene product (Rb) into G1 cells can block cell cycle progression (*4*). Thus, the elucidation of the molecular mechanism of Rb action should illuminate the process of normal cell growth control as well as the steps involved in oncogenesis.

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A major advance in the search for Rb function was the finding that the Rb protein is a target for the oncogenic products of the DNA tumor viruses. Initial studies demonstrated that the adenovirus E1A (early region 1A) protein forms a complex with Rb that is dependent on sequences in the E1A protein important for E1A oncogenic activity (*5*). The SV40 T antigen (*6*) and the human papillomavirus (HPV) E7 protein (*7*) also form complexes with Rb. Again, the formation of these complexes requires viral protein sequences that are also necessary for oncogenic activity. Thus, the interaction of these viral proteins with Rb would appear to be an important aspect of their oncogenic capacity, achieving an inactivation of Rb function equivalent to a deletion or mutation of *RB1*. Nevertheless, the mechanism of Rb action, and the identification of cellular targets for Rb action, remained unclear.

A variety of recent analyses have demonstrated a physical interaction between the Rb protein and the cellular transcription factor E2F (*8–10*) or a factor termed DRTF1 (*11*) that may be related or identical to E2F. Although identified in the context of adenovirus early region 2 (E2) gene transcription, E2F is important for the transcription of cell cycle–regulated genes such as c-*myc* (*12*, *13*) and the gene that encodes dihydrofolate reductase (DHFR) (*14*). The interaction of Rb, as well as a related protein termed p107, with E2F appears to control the transcriptional activating capacity of E2F (*15*, *16*).

The identification of E2F as a target for Rb is the result of a convergence of two distinct lines of investigation into the mechanism of action of E1A. Related studies have shown that E2F is also involved in cell cycle–regulated interactions, thus placing E2F in a broader role in cell cycle events. Most importantly, these studies have provided a mechanism of action for the DNA tumor virus oncoproteins and a functional link between the action of a tumor suppressor protein and the viral oncoproteins.

Two Approaches to Define a Mechanism of E1A Action

The *E1A* gene encodes a regulatory activity essential for the activation of the early pattern of viral gene expression (*17*, *18*) (Fig. 1A). Previous experiments established that *E1A*, along with the viral *E1B* gene, was also responsible for the oncogenic activity of the adenovirus (*19*).

One approach to investigating the function of E1A focused on its role in transcriptional activation. The *E2* gene is one of six viral transcription units that are activated during the early phase of viral infection in response to E1A (*20*) (Fig. 1A). The cellular transcription factor E2F was considered a likely target for E1A activation of E2 transcription for several reasons. (i) E2F is a DNA binding protein that recognizes the duplicated sequence element TTTCGCGC within the E2 promoter, and E2F DNA binding activity is elevated after adenovirus infection (*21*). (ii) The duplicated E2F sites within the E2 promoter are critical for E1A-induced transcription (*22*). (iii) A single E2F site can confer E1A regulation to a test promoter (*23*).

Although E2F specifically recognizes and binds to sites within the E2 promoter, this interaction is unstable. The stability of binding is markedly enhanced by the interaction of a 19-kD product of the viral *E4* gene with E2F (Fig. 2A). The E4 protein enables E2F to bind cooperatively to the two adjacent E2F sites in the E2 promoter, generating a very stable DNA-protein com-

plex (*24*) (Fig. 2A). Although the interaction of E2F with the E2 promoter is sufficient to stimulate transcription, transcription is further increased by the stable association of the E2F-E4 complex (*25*).

Subsequent experiments demonstrated that E2F was found in a heteromeric complex with other cellular proteins, which prevented its interaction with E4 (*26*) (Fig. 2B). With the development of an in vitro assay for E1A function, it was found that the E1A protein could disrupt these heteromeric complexes, releasing E2F to interact with E4 (*26*) (Fig. 2C). Analysis of a variety of E1A mutants for their ability to dissociate E2F complexes demonstrated that E1A sequences in the CR1 and CR2 domains of E1A (Fig. 1B) were required for the dissociation of the complexes (*27*). This activity was independent of the CR3 sequences previously shown to be important for the majority of early adenovirus transcription activation (*20*); thus, the ability of E1A to dissociate the E2F complexes is not correlated with the activation of the majority of early viral transcription (*28, 29*). This activity is, however, correlated with the activation of E2 transcription and with the oncogenic activity of E1A, thus linking a biochemical activity of E1A to the mechanism of E1A-mediated oncogenesis.

A second approach to study E1A function focused on its role in oncogenesis. Attention was directed at identifying proteins that bound to E1A as a way of finding potential cellular targets (Fig. 3A) (*30*). A major advance came with the discovery that one of the cellular proteins that complexed with E1A was the product of the retinoblastoma susceptibility gene (*RB1*) (*5*). This suggested that the E1A gene product was achieving the equivalent of a mutational event at the *RB1* locus through the inactivation of Rb function by complexing to the Rb protein.

This conclusion was further supported by the realization that the E1A-Rb interaction was dependent on sequences in the CR1 and CR2 domains of E1A that were essential for the transforming function of E1A (*31*) (Fig. 1B). Moreover, Rb sequences that were essential for interaction with E1A coincided with the positions of inactivating Rb mutations found in tumor cells (*32*). Thus, a direct correlation was made between the function of E1A as an oncoprotein, the function of Rb as a tumor suppressor protein, and the physical interaction between the two proteins. Moreover, not only E1A complexed with Rb but so did SV40 T antigen (6) and E7 of HPV (*7*), two proteins that share amino acid sequences with E1A (Fig. 1B). Thus, data emerged linking the action of these three viral transforming proteins to effects on a

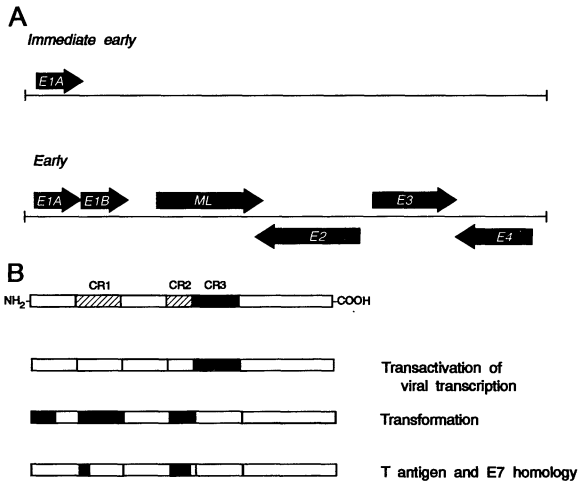

Fig. 1. Functional aspects of the adenovirus *E1A* gene. (**A**) The transcriptional program activated during the early stage of an adenovirus infection. In the absence of E1A function, E1A is the only transcription unit that is efficiently expressed. As a function of E1A, transcription of the remaining five early transcription units as well as the proximal half of the major late (ML) transcription unit is stimulated. (**B**) Domains of the E1A protein. The three regions of the E1A sequence that have been conserved during viral evolution are CR1, CR2, and CR3 (*70*); the functional domains of E1A that are involved in transcriptional activation and oncogenesis are indicated. Also indicated are the regions of E1A sequence that bear homology with sequence in SV40 T antigen and HPV E7 and that are involved in the binding to the Rb protein.

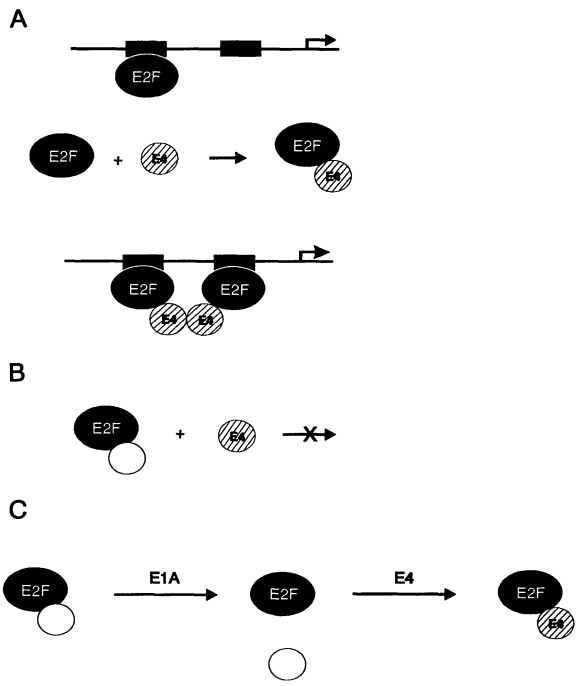

Fig. 2. Protein-to-protein interactions involving the E2F factor. (**A**) The interaction of a free E2F molecule with a recognition site in the adenovirus E2 promoter. Because of the unstable nature of the interaction, it is unusual for both sites to be occupied by E2F. The 19-kD E4 gene product can interact with E2F, forming a heteromeric protein complex. This complex can bind to adjacent sites in the E2 promoter in a highly cooperative fashion, forming a very stable DNA-protein complex (*24*). (**B**) In most cell types, E2F is complexed to other cellular proteins, preventing the interaction with E4 protein (*26*). (**C**) The adenovirus E1A protein can dissociate the E2F-containing complex, releasing E2F (*26*). This released E2F can combine with E4, forming the E2F-E4 heteromeric complex that can bind cooperatively to the E2 promoter.

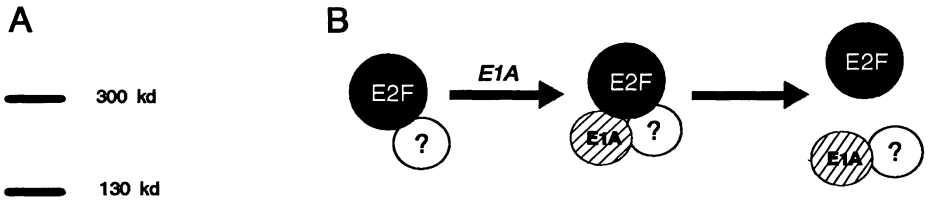

Fig. 3. Relationship of the interaction of E1A with cellular proteins and the dissociation of E2F complexes. (**A**) Schematic of the results of a typical immunoprecipitation experiment that identified the cellular proteins associated with E1A (*30*). The major proteins found by these assays are shown and include four proteins that have been cloned and identified: the Rb-related p107 protein (*71*), the p105 retinoblastoma gene product (*5*), the p60 cyclin A protein (*72*), and the p33 cdk2 kinase (*73*). (**B**) The process of E2F complex dissociation by E1A. The interaction of E1A with the complex as an intermediate in the dissociation process is speculative.

single cellular protein involved in the control of cell proliferation. Nevertheless, despite these observations and the realization that the interaction between these viral oncoproteins and the cellular Rb protein was undoubtedly of relevance in the development of the oncogenic phenotype, the consequence of these interactions remained unclear. In short, the normal role of Rb was not established.

E2F Complex Dissociation and Rb Binding: The Same E1A Activity

Although the precise mechanism for the dissociation of E2F complexes remains to be determined, the general scheme is as depicted in Fig. 3B: an initial E2F-containing complex is disrupted by E1A, which results in the release of free E2F. One possible outcome is the formation of a stable complex with E1A bound to the protein or proteins originally bound to E2F. Strikingly, such a complex would be the same as observed in the E1A coimmunoprecipitation experiments (Fig. 3A). Moreover, the ability of E1A to dissociate E2F complexes was dependent on E1A protein sequences that were essential for the interaction with many of the cellular proteins. Thus, it seemed possible that the proteins identified as E1A binding proteins might have been transferred onto E1A from E2F.

Reagents that could detect several of the E1A-associated proteins facilitated experiments to determine if one or more of these proteins were components of the E2F complexes. Initial experiments demonstrated the presence of the Rb protein in association with E2F (*8*) or DRTF1 (*11*). Additional assays have shown that the majority of the E1A-binding proteins (Fig. 3A) are in complexes with E2F (*33–37*). Thus, the general model depicted in Fig. 3B appears to be accurate.

An alternate path to elucidating the function of Rb came to the same conclusion: E2F was a cellular cohort of Rb. It was found that an Rb-containing protein complex is capable of sequence-specific DNA binding (*10*). Analysis of the DNA sequences that were selectively bound by the complex demonstrated that the vast majority were similar to the E2F recognition sequence TTTCGCGC, which implies that one of the cellular proteins complexed to Rb was E2F or a protein with related DNA binding specificity.

Additional evidence for an E2F-Rb interaction came from studies that identified an activity in extracts of mouse L cells that inhibited the DNA binding capacity of E2F (*27*). This inhibition was reversible by treatment with either the detergent deoxycholate or E1A, which suggests the formation of a complex with E2F. Purification of this E2F inhibitory factor demonstrated that Rb was also a constituent of the activity (*9*). Thus, an apparent paradox arose: Rb was a component of a complex with E2F that clearly could bind to DNA, but Rb was also a component of an activity that inhibited DNA binding by E2F. A resolution of the paradox is unknown, but one recent result suggests an explanation. The addition of a GST (glutathione-S-transferase)-Rb fusion protein to a partially purified preparation of E2F can reconstitute the E2F-Rb complex in cell extracts (*15, 38*). However, if the E2F preparation is further purified, Rb addition no longer generates the complex, which suggests that a third component is necessary for complex formation. Analysis of the E2F inhibitory activity suggested that it contained other polypeptides besides Rb (*9*). Thus, Rb has the capacity to recognize and interact with E2F, but whether this complex can bind to DNA or is blocked in DNA binding may depend on the nature of additional components.

Although E2F appears to be a target for Rb action, it may not be the only target. In vitro binding assays have revealed an interaction between the c-Myc protein and Rb (*39*), although the significance of the interaction is unclear because a complex has not been detected in cell extracts. Several additional proteins have been identified that bind to Rb (*40–42*), but again, the relevance is unclear because of the lack of evidence for an in vivo association.

Table 1. E2F sites in promoters of cellular genes. With the exception of the c-*myb* promoter, the position of the E2F binding site in the indicated promoter is given relative to the transcription initiation site. For c-*myb*, the position is relative to the ATG initiation codon because there are multiple transcription initiation sites. The ability of E2F to bind to the indicated sequence elements has been demonstrated in competition binding assays (*64, 68*).

Gene	Sequence	Position	Reference
DHFR	TTTCGCGC	+2/+9	(*14, 63*)
	TTTGGCGC	+13/+6	
Thymidine kinase	TTTGCCGC	−105/−112	(*64, 65*)
DNA polymerase α	TTTGGCGC	−128/−135	(*64, 66*)
c-*myb*	TTTGGCGG	−278/−271	(*67, 68*)
c-*myc*	TTTCCCGC	−58/−65	(*12, 13*)
cdc2	TTTCGCGC	−128/−121	(*52*)
N-*myc*	TTTCGCGC	−130/−137	(*68, 69*)
	TTTGGCGC	−142/−134	
	TTTGGCGC	−126/−119	
Adenovirus E2	TTTCGCGC	−67/−60	(*21*)
	TTTCGCGC	−36/−43	

The Significance of the E2F-Rb Interaction

Several observations suggest that the interaction of Rb with E2F is functionally significant with respect to the action of Rb as a tumor suppressor. (i) Little, if any, E2F-Rb complex is found in cells that produce a nonfunctional Rb protein; instead, there is an abundance of free E2F (*8, 43*). (ii) The E2F-Rb complex is disrupted by each viral oncoprotein previously shown to bind to Rb (*43*). Thus, T antigen, E1A, and E7 dissociate the E2F-Rb complex, and this dissociation is dependent on domains of these viral proteins that are essential for their oncogenic activity. (iii) The disruption of Rb function in human cervical carcinoma cells, either by the HPV E7 protein or by mutation of *RB1* (*44*), coincides with the loss of the E2F-Rb interaction (*43*). (iv) The ability of Rb to reconstitute the E2F-Rb complex in an in vitro assay is dependent on Rb sequences that are also required for Rb to suppress cell growth (*4*). Thus, the loss of Rb function is associated with a loss of the E2F-Rb interaction, which strongly suggests that E2F is a functionally relevant target for Rb.

Additional E2F Interactions That Are Regulated by the Cell Cycle

Rb is not the only cellular protein that interacts with E2F. Extracts from synchronized NIH 3T3 cells contain at least two other E2F-containing complexes (*33*). One complex accumulates during the G1 phase of the cell cycle and then disappears at the end of G1. A second, distinct complex appears at the beginning of the S phase, accumulates during the S phase, and then disappears in G2 or mitosis. When cells finish mitosis, the G1-specific complex reappears. The S phase–regulated E2F complex contains the cyclin A protein (*33*). Although in vitro reconstitution assays show that cyclin A and Rb can interact in the same DRTF1 complex (*34*), such a complex has not been detected in vivo. Assays of E2F have shown that cyclin A and Rb are components of distinct complexes in vivo (*8, 35–37*). The S phase–specific E2F–cyclin A complex also con-

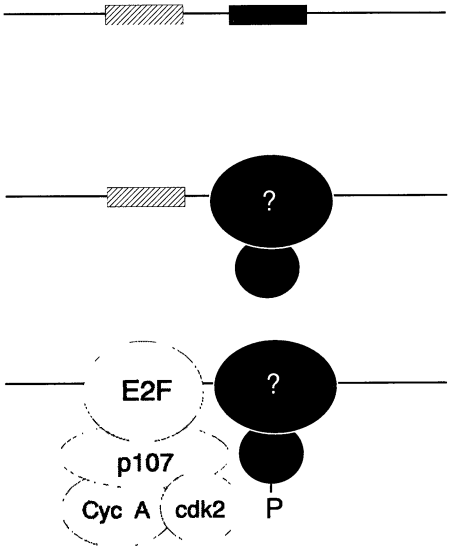

Fig. 4. The potential functional role of E2F in association with the cyclin A–cdk2 kinase. The schematic depicts a segment of DNA containing an E2F recognition sequence and an adjacent binding site for an unknown protein. Upon binding of the E2F-p107-cyclin A–cdk2 complex, phosphorylation of the adjacent protein might take place. Cyc A, cyclin A.

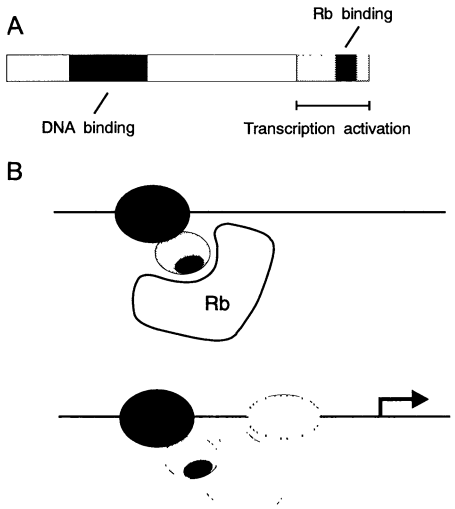

Fig. 5. Functional domains and interactions of E2F. (**A**) The functional domains identified in the *RBP3* and *RBAP1* gene products (*57, 58*). The DNA binding domain includes the amino acid sequence from residues 89 to 191; the transcription activation domain includes the sequence from residues 368 to 437. The sequence essential for Rb binding includes residues 409 to 426. (**B**) The interaction of the E2F-Rb complex with a promoter. (**Top**) Because of the interaction of Rb with E2F, the transcriptional activation capacity of E2F may be masked. (**Bottom**) In contrast, E2F free of Rb is able to interact with other transcriptional components and thus to stimulate transcription.

tains the Rb-related p107 protein as well as the p33 cdk2 kinase (*35–37*). Other experiments have shown that cyclin A likely interacts directly with the p107 protein (*45*).

The previously identified, G1-specific E2F complex has now been shown to contain the same Rb-related p107 protein, and reconstitution experiments suggest that p107 may be the only additional component (*16*). The situation may be even more complicated because other experiments have identified an E2F complex, also in G1, that contains p107 together with the cyclin E polypeptide and the cdk2 kinase (*46*). Thus, it seems likely that p107 makes direct contact with E2F with several possible outcomes. The resulting E2F-p107 complex can remain or the p107 protein may serve to bring either the cyclin E–cdk2 or the cyclin A–cdk2 complex into association with E2F.

The Functional Consequence of the E2F Interactions

The effect that the interaction of these various proteins has on E2F function has been investigated by transient transfection assays to score for the transcriptional activating capacity of E2F. Such assays demonstrate that co-transfection of an Rb-expressing plasmid inhibits E2F-dependent transcription and that this is correlated with the capacity of Rb to interact with E2F (*15*). More recent experiments have shown that co-transfection of a p107-expressing plasmid also inhibits E2F-dependent transcription (*16*). Thus, it appears that the transcriptionally active form of E2F may be the form that is free of interactions with cellular proteins such as Rb or p107.

Although initially identified as promoting adenovirus E2 transcription (*47*), E2F is clearly important for cellular gene transcription. E2F is likely to be involved in the transcription of the gene that encodes DHFR (*14*) and may regulate other genes that have E2F binding sites in their promoters (Table 1). Many of these genes are activated in late G1 as cells reenter the growth cycle, and they encode proteins required for DNA synthesis. Indeed, activation of DHFR transcription in late G1 is dependent on the E2F sites found in the promoter (*48*). Thus, the transcriptional activating capacity of E2F may be regulated by interactions with cellular proteins, resulting in the release of active E2F at late G1 and the concomitant activation of the DNA synthesis genes.

The presence of only the underphosphorylated form of Rb in the E2F-Rb complex (*8*) suggested that this interaction might be restricted to G0 or G1 because this is when underphosphorylated Rb predominates (*49*). Then, as Rb becomes phosphorylated as cells progress into the S phase, E2F might be released to activate transcription. However, recent experiments demonstrate that although the E2F-Rb complex is detected in G1, it persists into the S phase (*16, 37*). Because only the underphosphorylated form of Rb is found in the complex, it would appear that E2F must be associated with the small amount of underphosphorylated Rb that persists beyond the G1/S boundary. A role for the Rb protein in the regulation of E2F at G1/S therefore seems unlikely. There is evidence for a G1/S-regulated interaction involving Rb that has been obtained from cellular localization assays of Rb, but the nature of the interaction, including the Rb partner, has yet to be characterized (*50*). Because of its G1-specific association with E2F, a better candidate for regulation of E2F during G1 is the p107 protein.

If the interaction of Rb with E2F inhibits E2F transcription activity but is not involved in G1/S-regulated transcription, what is the role of the E2F-Rb complex? One possibility is a direct negative regulation of certain target genes such as c-*myc* and *cdc2* because E2F binding sites in the promoters of these genes have been implicated in repression by Rb (*51–53*). In this context, the interaction of Rb with E2F would not simply inhibit the transcriptional function of E2F but would create a dominant repressor complex, a result consistent with recent experiments that demonstrate that an E2F site can be a negative element dependent on the presence of Rb (*54*).

The recent finding that the S phase–regulated E2F–cyclin A complex also contains both the Rb-related p107 protein and the $p33^{cdk2}$ protein kinase (*35–37, 55*), thus forming a cyclin-dependent protein kinase with sequence-specific DNA binding activity, suggests an altogether distinct role for E2F (Fig. 4). Although this complex may be another transcriptionally inactive form of E2F, it could also play an active role in S phase events if E2F were to serve as a chaperone or co-factor for the cdk2 kinase. The targeting of a cyclin-dependent protein kinase to DNA could stimulate the local phosphorylation of other DNA-bound proteins and, in so doing, alter their function. A variety of assays suggest a role for cyclin A and cdk2 in the regulation of S phase and M phase events (*56*). These activities may be a result of cyclin A and cdk2 that are associated with E2F.

The Cloning of E2F

Taking advantage of the ability of Rb to specifically interact with a variety of cellu-

lar proteins, researchers isolated a series of clones from λgt11 expression libraries that encode proteins that interact with Rb (*41, 42*). Although the identity of most remains unknown, one encodes E2F. In fact, two laboratories isolated an identical clone, as indicated by sequence identity, independently and simultaneously (*57, 58*). Several lines of evidence support the conclusion that this clone, termed either *RBP3* or *RBAP1*, does encode an E2F activity. Antibodies directed against the cloned product are able to immunoprecipitate E2F binding activity from cell extracts. Immunoprecipitation of cell extracts with an Rb antibody co-precipitates the product of the cloned gene, as measured with the specific antibody. The expressed protein can bind to an E2F recognition site and can interact with the Rb protein as well as with the adenovirus E4 gene product. Finally, expression of the cloned product in transfected cells can stimulate transcription in an E2F site–dependent manner.

The initial functional studies of the E2F clone have defined domains responsible for DNA binding, transcriptional activation, and Rb binding (Fig. 5A). The DNA binding region appears to be a basic helix-loop-helix domain, which suggests that dimer formation may be involved. Although previous biochemical analyses of E2F did not provide evidence that the protein functioned as a dimer, there is no evidence opposing this notion. The COOH-terminal 69–amino acid residues appear to constitute a transcriptional activation domain that exhibits a characteristic acidic motif. Interestingly, the Rb binding domain is embedded within the transcription activation domain, consistent with the possibility that the binding of Rb to E2F may physically block the activation domain and interfere with the transcriptional activation capacity of E2F (Fig. 5B). The Rb interaction domain lacks the characteristic Leu-X-Cys-X-Glu (where X is any amino acid) motif found in the viral oncoproteins E1A, T antigen, and E7 and also in several other cellular proteins that bind to Rb (*43*). Thus, the nature of the interaction of E2F with Rb may be different from the nature of the interaction of these other proteins.

It appears that this clone does not represent all of the E2F activity within the cell but instead may be a member of a larger family. Although biochemical assays for E2F have not provided evidence for multiple species, at least one additional clone has been obtained by reduced stringency screening with the DNA binding domain of the original E2F clone (*59*). The encoded protein also exhibits E2F-specific DNA binding, but it is not yet clear if it is able to interact with Rb.

Speculations

It is important to consider these events in the context of the role of proteins such as E1A, T antigen, and E7 in the growth of the respective DNA tumor viruses. Although the ability of E1A, T antigen, or E7 to interact with Rb is generally considered in the context of oncogenic transformation, these viruses have not evolved to transform cells; rather, it is probably safe to suggest that the purpose of the common ability of these viral proteins to disrupt E2F complexes and release transcriptionally active E2F is to enhance replication of the respective viruses.

Although the adenovirus E2 promoter clearly utilizes E2F—indeed, a second viral gene (E4) has evolved to make the most efficient use of E2F—there are no E2F sites within the SV40 genome or the papillomavirus genome (*60*). Thus, these other viruses do not target E2F for viral transcription; they may instead use E2F in order to stimulate quiescent cells into the S phase. Although each virus encodes proteins required specifically for viral DNA replication, efficient viral replication requires an elevation of the appropriate substrates and other cellular activities necessary for DNA synthesis (*61*). As mentioned previously, a variety of cellular genes that encode proteins necessary for DNA synthesis possess E2F sites within promoter regulatory sequences, and the E2F sites in the DHFR promoter are essential for activation at the late G1 stage (*48*). Moreover, experiments have demonstrated that DHFR and thymidine kinase are activated in both adenovirus- and SV40-infected cells (*62*). Therefore, the common function of E1A, SV40 T antigen, and E7 in a lytic infection could be to stimulate cells into the S phase through the dissociation of E2F regulatory interactions. Perhaps adenovirus has simply taken further advantage of this situation through the evolution of an additional activity (E4) that can direct the released E2F to the viral E2 promoter.

If E2F is an important target for the regulatory action of Rb as well as proteins such as p107, cyclin A, and cdk2, it is possible that an alteration of the E2F coding sequence might have significant consequences on cell proliferation control. For example, a mutation producing an E2F protein that could bind to DNA and stimulate transcription but that was unable to interact with Rb might be expected to have a phenotype similar to a loss of Rb. Thus, under some circumstances E2F could be oncogenic. It is also possible that overproduction of E2F, as a result of gene amplification or altered regulation of expression, could produce the same result by overwhelming the mechanisms that normally control E2F activity.

Finally, if one of the roles attributed to cyclin A or cdk2 during the S phase is dependent on the interaction with E2F, an alteration of E2F that prevented the formation of this complex might lead to S phase abnormalities. In sum, we might expect to find mutations in the *E2F* gene that correlate with altered cellular phenotypes.

Studies of the interaction of the E2F transcription factor with Rb and other regulatory proteins such as cyclin A have succeeded in uniting two diverse lines of investigation: oncogenesis and transcriptional control. Undoubtedly, more is to be learned concerning the mechanisms by which the Rb protein controls cell growth and about the significance of the interaction of E2F with the cyclin A–cdk2 kinase complex. Nevertheless, the identification of these interactions represents an important step forward in our understanding of the role of these proteins in the control of cellular proliferation and the manner in which this control is lost as a consequence of the action of viral oncoproteins.

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