Trans-activation of the adenovirus E2 promoter by human papillomavirus type 16 E7 is mediated by retinoblastoma-dependent and -independent pathways

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In common with the adenovirus E1A and simian virus 40 large T oncoproteins, the E7 protein of human papillomavirus (HPV) type 16 interacts with the retinoblastoma (Rb) tumour suppressor protein (pRb). The functional importance of this interaction for HPV-16 E7 protein was investigated by analysis of the trans-activating function of E7 at the adenovirus E2 promoter in a set of breast tumour cell lines. Trans-activation by HPV-16 E7 in two pRb-deficient cell lines demonstrated that pRb is not essential for E7-mediated trans-activation, but reconstitution of Rb expression indicated the existence of an Rb-mediated pathway of E7 trans-activation. This pathway results from suppression by E7 of a trans-repressing function encoded by the Rb gene. The E7 protein is shown to be capable of interacting in vivo with the Rb-related protein p107. Furthermore, analysis of a fusion construct between the amino terminus of Rb and the carboxy terminus of p107 suggests that, in common with pRb, the p107 protein trans-represses the adenovirus E2 early promoter. Therefore it is proposed that the pRb-independent pathway of E7 trans-activation is a consequence of the suppression of trans-repression by p107.

The human papillomaviruses (HPVs) have been extensively investigated as a potential causative agent of human warts, both benign and malignant. A subgroup of these viruses, including types 6, 11, 16 and 18, infect the cervical epithelia. Each of these types has been found in both low and high grade lesions, but epidemiological analysis of cervical biopsies indicates that types 6 and 11 are associated mainly with low grade lesions and types 16 and 18 are associated with lesions having a higher risk of progression (de Villiers, 1989). HPV-16-derived sequences are commonly found integrated within the cellular genome of cervical cancer biopsies and derived cell lines and analysis of the pattern of integration of the virus reveals specific retention of sequences encoding the E6 and E7 open reading frames (ORFs) but disruption of viral regulatory functions (Baker et al., 1987; Johnson et al., 1990). These data suggest that deregulated expression of E6 and E7 is an important causative event for HPV-associated cervical cancer.

In addition to its transforming activity, E7 trans-activates the adenovirus E2 early promoter and stimulates cellular DNA synthesis (Phelps et al., 1988). Mutational analysis of the E7 gene has demonstrated partial resolution of its trans-activating and transforming activities, but no mutations abolish one of these activities without impairing the other (Barbosa et al., 1990; Choo & Chong, 1993; Crook et al., 1991; Scheffner et al., 1991). These proteins, in common with the analogous proteins encoded by the DNA tumour viruses, simian virus 40 (SV40) and adenovirus, bind with and presumably inactivate the p53 and retinoblastoma (Rb) tumour suppressor protein (pRb). SV40 large T antigen, adenovirus Elb and E6 bind with p53, whereas SV40 large T antigen, adenovirus E1a and E7 bind with pRb (Dyson et al., 1989; Scheffner et al., 1990; Werness et al., 1990). Inactivation of these tumour suppressor proteins by genomic rearrangements or mutation of their ORFs is a frequent occurrence in a wide spectrum of cancer types, suggesting that loss of their function is an important step towards the cancerous state (Baker et al., 1989; Friend et al., 1987; Takahashi et al., 1989; Yokota et al., 1988). However, in HPV-containing tumours these changes may be much less frequent, suggesting that the same function may be fulfilled by binding of E6 and E7 (Choo & Chong, 1993; Crook et al., 1991; Scheffner et al., 1991).

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Edmonds & Vousden, 1989; Storey et al., 1990; Watanabe et al., 1990). This is in contrast to the situation with the adenovirus E1a gene (Moran et al., 1986; Schneider et al., 1987), which otherwise shares many similarities with E7. It is therefore likely that the pathway by which E7 induces transformation is similar or identical to the pathway by which it trans-activates and that interaction of E7 with the same set of cellular proteins endows the gene with both activities. Therefore, the potential involvement of pRb in the mechanism by which E7 acts at the adenovirus E2 promoter was investigated.

Eight breast tumour cell lines were selected in order to analyse the trans-activating function of E7. Previous Southern blot analysis has shown that the Rb gene is deleted in the cell lines MDA-MB-468 and BT549 but is apparently normal in the other six cell lines (T'Ang et al., 1988). To confirm and extend these results to the protein level, crude lysates were prepared from each cell line and analysed by immunoblotting with a pRb-specific monoclonal antibody. As shown in Fig. 1, pRb was detected in all six of the putatively Rb gene-positive cell lines but in neither of the lines carrying deletions within the Rb gene. The protein visualized in the Rb-positive cell lines is presumed to be wild-type since, based on previous studies, it is unlikely that it is mutated without abolishing the characteristic set of bands produced by differential phosphorylation (Templeton et al., 1991).

An expression plasmid for HPV-16 E7 was reconstructed by PCR amplification of the E7 ORF from a previously constructed pJ4Ω-based plasmid to remove extensive 3′ flanking sequences (Storey et al., 1988). The primer pair AGCTCAATAAAAGAGCCC (5′, anneals within vector) and GGATTCTATGGTTTCTGA-GAACAGATGGG (3′, anneals with E7) were used for the amplification, and the fragment was cloned using the BamHI and EcoRI restriction sites. This yielded an insert in the pJ4Ω vector consisting of the E7 ORF preceded by a C residue derived from the initial cloning. The E7-responsive reporter plasmid E2-CAT, which contains the adenovirus E2 early promoter directing transcription of the chloramphenicol acetyltransferase (CAT) gene, has been previously described (Murthy et al., 1985). A β-galactosidase-expressing internal control plasmid was constructed by transferring the β-galactosidase coding region from pBl-βgal (gift of Dr N. Jones) to pJ4Ω using the HindIII and BgII restriction sites. Plasmids were purified by alkaline lysis and banding in caesium chloride/ethidium bromide gradients then transfected by calcium phosphate coprecipitation. CAT and β-galactosidase assays were performed using standard methods. All data were reproduced in at least three independent experiments.

Analysis of the trans-activating function of E7 in the eight breast tumour cell lines clearly demonstrated that E7 is able to trans-activate in either the presence or the absence of pRb (Fig. 2). The degree of trans-activation elicited by E7 varied between the cell lines, but that observed in the pRb-deficient cell lines was comparable with that in the pRb-positive cell lines. The differing trans-activation potential in the various cell lines is not a simple consequence of transfection efficiency since this is comparable (as judged by expression from the internal control plasmid) for each cell line with the exception of MCF7, which takes up plasmid 10-fold more efficiently.
It is most likely that the cause of variation in E7 function between the cell lines is related to the numerous genetic differences incurred during transformation. Experiments analogous to those presented in Fig. 2 have been performed using lipofection rather than calcium phosphate coprecipitation to introduce the DNA into the cells (data not shown). In these experiments we consistently observed negligible trans-activation in the pRb-deficient cell lines MDA-MB-468 and BT549, illustrating that the two methods are not interchangeable.

Although it was clear that trans-activation by E7 was not mediated exclusively by its interaction with pRb, it was possible that this interaction could represent one of several pathways by which E7 activates the adenovirus E2 promoter. This model would suggest that the degree of E7 trans-activation observed in a particular cell line should be affected by its pRb status, but direct comparison between cell lines might not be informative because of other uncharacterized differences. Therefore, a pRb expression plasmid was constructed, by transferring the BamHI Rb gene-containing fragment from pJ3ΩRb-c (Robbins et al., 1990) to pJ4Ω, to analyse its effect on E7-mediated trans-activation. Expression from the same promoter as used for E7 was considered desirable to increase the probability of the two proteins being expressed at similar levels. The analysis presented of the effects of reconstituted pRb expression on E7 activity was performed in the cell line BT549. The cell line MDA-MB-468 has been analysed less extensively giving essentially the same results (data not shown). An important consideration in designing these experiments is that pRb activity is regulated during the cell cycle so care must be taken to avoid potential cell cycle-dependent artefacts in the data. The elapsed time between transfection and harvesting of cells might be important in this experiment for two reasons. First, if the transfected plasmids predominantly enter cells at a specific stage of the cell cycle it is possible that cell cycle-dependent regulation might affect the result at different harvesting times. Second, correct function of pRb might be dependent on passage through a certain phase of the cell cycle or require an extended time period. Therefore, these transient transfections were harvested at multiple time points. As shown in Fig. 3, expression of exogenous pRb suppressed the activity of the E2-CAT reporter plasmid. This suppression was relieved by E7, especially at later time points, resulting in an increase in the degree of activation by E7 in the presence of pRb compared to that in its absence. The lag in suppression of the effects of pRb by E7 may reflect the need for an equilibrium to be established between the levels of E7 and pRb. These results are specific for the E2-CAT reporter plasmid since the activity of the internal control pJ4Ω/βgal plasmid did not differ between data sets. The internal control also eliminates the possibility that the apparent interaction is the consequence of activity of E7 or pRb on the promoter region of pJ4Ω. Thus, one pathway of trans-activation by E7 is inactivation of trans-repression by pRb, but there are other, pRb-independent pathways.

In the above experiment, fixed doses of pRb and E7 were used. However, to demonstrate whether the function of pRb is fully or partially suppressed by interaction with E7 it is necessary to transfect an excess of E7. This is best achieved by a dose–response analysis of relief of pRb-mediated trans-repression by increasing amounts of E7. It was not possible to detect pRb and E7...
protein expressed in these transient transfections, probably because only a small fraction of the cells take up DNA, but it is reasonable to suppose that the level of protein expressed is proportional to the amount of expression plasmid transfected. It was found that the activity of the E2-CAT reporter plasmid in the pRb-deficient cell line BT549 was reduced by expression of pRb, but that as increasing amounts of E7 were transfected the activities with and without exogenous pRb converged, not differing significantly at the highest dose of E7 transfected (Fig. 4). This is strong evidence that E7 can completely inactivate the function of pRb.

Given that the pRb-mediated trans-repression is absent in the pRb-deficient cell lines BT549 and MDA-MB-468, it might be anticipated that their basal level of expression from the E2 promoter should be higher than in the other cell lines used. To compare this, the activities need to be standardized for transfection efficiencies. However, using different internal control reporter plasmids demonstrated that their relative activities varied between the cell lines, so a simple standardization would not be valid. In addition it is noted that there are multiple elements in the E2 promoter (Fig. 5), the activity of any of which may vary between the cell lines owing to differences in trans-acting factors. In particular we note that whereas the activity of E2-CAT is comparable in MCF7 and T47D cells, the activity of Δ74-CAT is an order of magnitude lower in the former (standardized to the pJ4Ω-βgal internal control plasmid).

Previous work has indicated that the elements within the adenovirus E2 promoter mediating trans-activation by E7 are the E2F sites (Phelps et al., 1991). The analysis described in the previous sections identifies two independent pathways of E7 trans-activation which might be mediated entirely by the E2F sites or, whereas the E2F sites might be required for both, one pathway might in addition depend on other elements of the E2 promoter. This latter possibility would explain the reduction in the level of trans-activation of the E2 promoter when elements which alone are insufficient to mediate E7 trans-activation are deleted (Phelps et al., 1991). Two modified reporter plasmids have been analysed to determine these possibilities: the construct LS-74/85 contains a BamHI linker insertion within the activating transcription factor (ATF) site (Murthy et al., 1985) and in Δ74-CAT this site has been used to remove all E2-derived sequences upstream of the E2F sites.

Fig. 4. Analysis of concentration dependence of basal and reconstituted trans-activation by E7 in BT549 cells. Cells were transfected with 5 μg pJ4Ω/pJ4ΩRb, 5 μg E2-CAT, 2 μg pJ4Ω/βgal and pJ4ΩΔE7/pJ4Ω as indicated. CAT activity was determined 60 h after transfection. Data are expressed relative to control; error bars indicate the s.d. of the data.

Fig. 5. The factor binding elements (ATF/E2F) identified within the adenovirus E2 early promoter and the major/minor transcription start site are shown diagrammatically with the sequence encompassing the former expanded below. The reporter E2-CAT contains the wild-type sequence in this region, LS-74/85 is disrupted by linker insertion within the ATF site and Δ74-CAT is deleted for all E2-derived sequences upstream of the E2F sites.
which it interacts. An obvious candidate is the pRb-related protein p107, for which a partial, C-terminal sequence is known (Ewen et al., 1991). To assess this possibility, experiments were performed to determine whether E7 interacts with p107 and whether p107, in common with pRb, trans-repressed the adenovirus E2 promoter. This latter analysis was considered particularly important since the function of p107 might be either similar or antagonistic to that of pRb. Using radio-labelled cell extracts, we were able to demonstrate an interaction between bacterially expressed E7 protein and a cellular protein of Mr approximately 107K in both pRb-containing and -deficient cell lines (data not shown).

However, the interpretation of these data is limited since the interaction demonstrated might not occur in vivo and the 107K protein might not be p107. To circumvent these problems, the potential of E7 to interact with p107 in vivo was assessed using a Gal4/VP16-based two-hybrid approach (Ma & Ptashne, 1988).

The E7 ORF was fused with the Gal4 DNA-binding domain by transferring the HindIII–BamHI Gal4-containing fragment from Gal4VP16 (Martin et al., 1990) to pJ4ΩE7, then infilling the BamHI site to obtain fusion in the correct reading frame. The plasmids VP16-Rb and VP16-107 were constructed using fully sequenced PCR-derived products as follows. The VP16 domain was PCR-amplified from Gal4VP16 using the primers GGAAGCTTGCCATGACGGCCCCCCCG-ACCGATGTCAGC (5') and GGGATCCTGGGGTTGGAGACCGCCCGCCG-ACCGATGTCAGC (3'). The 5' primer adds a HindIII restriction site and an initiation codon in good context to the VP16 fragment and the 3' primer adds a BamHI restriction site and a short, flexible linker which ensures fusion with the Rb and p107 genes in the correct reading frame. The Rb fragment was derived by PCR amplification of pJ3ΩRb-c with the primer pair GGGATCCTGGGGTTGGAGACCGCCCGCCG-ACCGATGTCAGC (5') and TCCATTTATTTTGAGACCGCCCGCCG (3'), hybridizes to vector) and was cloned by digesting with BamHI and BglII. The p107 fragment was derived by PCR amplification of cDNA with the primer pair GGGATCCTGGGGTTGGAGACCGCCCGCCG-ACCGATGTCAGC (5') and GGGAATTCTTAATGATTTGCTCTTTCACTG (3'). The 5' primer adds a HindIII restriction site and the 3' primer adds a BamHI restriction site.

Table 1. Trans-activation of modified promoters in BT549 cells*

<table>
<thead>
<tr>
<th>Reporter</th>
<th>Control</th>
<th>E7</th>
<th>Rb</th>
<th>Rb+E7</th>
<th>Basal</th>
<th>Reconstituted</th>
</tr>
</thead>
<tbody>
<tr>
<td>E2-CAT</td>
<td>1±0.18</td>
<td>5.24±0.99</td>
<td>0.44±0.11</td>
<td>4.17±0.20</td>
<td>5.2±1.9</td>
<td>9.3±2.8</td>
</tr>
<tr>
<td>LS-74/85</td>
<td>0.35±0.12</td>
<td>1.17±0.06</td>
<td>0.12±0.02</td>
<td>1.14±0.23</td>
<td>3.3±1.3</td>
<td>9.9±3.6</td>
</tr>
<tr>
<td>Δ74-CAT</td>
<td>0.35±0.13</td>
<td>0.69±0.21</td>
<td>0.07±0.02</td>
<td>0.33±0.08</td>
<td>2.0±1.3</td>
<td>3.5±1.8</td>
</tr>
</tbody>
</table>

* BT549 cells were transfected with 3 μg each of reporter, pJ4Ω/pJ4ΩE7 and pJ4Ω/pJ4ΩRb plus 1 μg internal control plasmid. Cells were harvested 60 h after transfection. Activity is expressed relative to control level for E2-CAT, ± 1 S.D.

Table 2. Two-hybrid analysis of E7 interactions*

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>VP16-Rb</th>
<th>VP16-107</th>
</tr>
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<tbody>
<tr>
<td>Gal4-E7</td>
<td>1.00±0.12</td>
<td>0.97±0.08</td>
<td>1.33±0.26</td>
</tr>
<tr>
<td>Gal4-VP16</td>
<td>0.83±0.14</td>
<td>149±47</td>
<td>157±25</td>
</tr>
</tbody>
</table>

* T47D cells were transfected with 3 μg each of Gal4 plasmid, VP16 plasmid and GsElb-CAT reporter plus 1 μg internal control plasmid. Levels of CAT are expressed relative to the double control, ± 1 S.D.

GGGGATCCACTCTCTTGGCATCAGCCCGCCCG (5') and GGGAATTCTTAATGATTTGCTCTTTTACTG (3') and was subcloned as a BamHI–EcoRI fragment (reconstructed from two PCRs to remove base changes introduced by PCR). We note that this amplified cDNA contains one non-silent base change (T to C at position 2383, changing the encoded amino acid from serine to glycine) compared with the previously reported sequence (Ewen et al., 1991), which was detected with cDNA prepared from both MCF7 and T47D cells. This is most probably a neutral polymorphism since it changes serine 795, an amino acid not conserved between p107 and pRb, the latter having aspartate at the equivalent position.

Transfection of Gal4-E7, VP16-Rb or VP16-107 alone did not stimulate the Gal4-responsive reporter GsElb-CAT. However, transfection of Gal4-E7 with either VP16-Rb or VP16-107 resulted in marked stimulation of GsElb-CAT (Table 2). Therefore, we conclude that E7 is able to interact in vivo with both pRb and p107. It is not clear whether the stronger trans-activation observed with Gal4-E7 plus VP16-107 than with Gal4-E7 plus VP16-Rb is a consequence of stronger interaction or better expression of the fusion protein, but we note that these data suggest the two-hybrid system should provide a good method of screening for other proteins which interact with E7.

The deduced sequence for p107 lacks the extreme amino terminus. However, two lines of evidence suggested that a fusion between the amino-terminal part of pRb and the carboxy-terminal region of p107 would function similarly to the authentic p107 protein. First,
the amino terminus of pRb is dispensable for its tumour suppressor activity (Qin et al., 1992). Second, the major difference identified between pRb and p107 both in vivo and in vitro is the interaction of the latter with cyclin A (Ewen et al., 1992). This interaction is dependent on the so-called spacer domain within the pocket region of p107 which lies within the cloned C-terminal region. To construct the Rb-107 fusion, cDNA encoding amino acids 1 to 372 of the Rb gene were amplified with the primer pair GGGATCCGTGGGAAGATTATATTCACCT (3') and the 5′ oligonucleotide used to construct pJ4Q16E7 and subcloned as a BamHI fragment. This was then fused with the p107 gene cDNA fragment encoding amino acids 252 to the stop codon, flanked by BamHI and EcoRI in the pJ4Q vector to give pJ4QRb-107. The fusion protein encoded replaces the domain of p107 upstream of conserved domain A with the equivalent region of pRb and therefore should be minimally disruptive structurally. Transient transfection analysis indicated that the fusion protein trans-represses the adenovirus E2 promoter to a similar extent to pRb but only in the pRb-deficient cell lines (Table 3). Using an internally initiated p107 protein, Schwartz et al. (1993) have also identified trans-repressor activity of p107 at the adenovirus E2 promoter. Therefore, we conclude that p107 is likely to mediate part or all of the pRb-independent pathway of E7 trans-activation. It is interesting that we only observe trans-repression by Rb and Rb-107 in the pRb-deficient cell lines (we note also that Schwarz et al. (1993) performed their analysis in the cell line C33A which is deficient in pRb, but they do not indicate whether repression was also observed in pRb-positive cell lines). That no trans-repression ensues when pJ4QRb-107 is introduced into pRb-positive cell lines might be a consequence of at least three factors. First, expression of the exogenous gene might be compensated for by down-regulation of the endogenous gene. This seems unlikely, however, since the exogenous gene is expressed from a strong retroviral promoter and is likely to enter the cell in multiple copies. Our pRb expression vector contains some 5′ Rb flanking sequences which might contain regulatory elements, but removal of these does not affect the trans-repressing behaviour (data not shown). Secondly, regulation of pRb is probably at the level of phosphorylation, so it is possible that the excess protein expressed is inactive. Thirdly, as the cell lines used are tumour-derived, it is possible that they have other alterations which block the function both of the endogenous and the exogenous gene. Since these changes would be expected only in pRb-positive tumour cell lines, the best cell lines in which to study the function of pRb are probably either deficient in pRb or non-transformed. The pRb-p107 fusion protein might only be trans-repressing in the pRb-deficient cell lines either because the protein is regulated like pRb as a consequence of the pRb-derived sequences or because the same mechanisms that block trans-repression by pRb also block trans-repression by p107 in the pRb-positive cell lines. It is likely that all the cell lines tested contain functional p107 based on our own observations (data not shown) and because no p107-deficient cell lines are known.

We have shown that E7 trans-activates the adenovirus E2 promoter by at least two pathways, one being dependent on pRb. Removal of all sequences upstream of the distal E2F binding site of the E2 promoter left both pathways intact, albeit at a reduced level. Since previous data indicate that the E2F sites are critical for E7-mediated trans-activation, it is likely that both pathways depend on the E2F sites (PHELPS et al., 1991). As the E2F site appears to encode a negative element, which silences adjacent promoters under some circumstances but a positive element when activated (WEINTRAUB et al., 1992), the reduction of trans-activation activity when the upstream sites are removed might be a consequence of removing an E7-independent positive element. This would explain both the reduction in background activity (removal of constitutively activating elements) and degree of inducibility by E7 (since E7 is able to convert E2F from a negative to a positive element but relief of the silencing effect is not observed) of the mutant promoters.

The transcriptional repressing activity of pRb due to its interaction with the E2F factor has been much studied recently (Bagchi et al., 1991; Chellappan et al., 1991; CHITTENDEN et al., 1991; Robbins et al., 1990). Although E2F was originally identified as a factor binding to the adenovirus E2 early promoter, it also binds to the promoter sequences of many S phase-specific cellular genes (Kovesdi et al., 1986). pRb binds to the E2F transcription factor, masking its trans-activating domain and converting the E2F site from a positive to a negative element (Helin et al., 1992; Kaelin et al., 1992; Weintraub et al., 1992). Gel shift experiments have demonstrated that several complexes are formed at the E2F binding site: a small complex probably represents free E2F and two larger complexes contain pRb and p107 (Shirodkar et al., 1992). Several lines of evidence suggest that the

### Table 3. Activity of Rb and Rb-107 expression plasmids*

<table>
<thead>
<tr>
<th>Cell line</th>
<th>pJ4QRb-c</th>
<th>pJ4QRb-107</th>
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<tbody>
<tr>
<td>MCF7</td>
<td>1:03±0.12</td>
<td>1:11±0.04</td>
</tr>
<tr>
<td>T47D</td>
<td>1:15±0.20</td>
<td>1:19±0.11</td>
</tr>
<tr>
<td>MDA-MB-468</td>
<td>0:56±0.13</td>
<td>0:61±0.08</td>
</tr>
<tr>
<td>BT549</td>
<td>0:33±0.05</td>
<td>0:54±0.05</td>
</tr>
</tbody>
</table>

* The indicated cell lines were transfected with 4 µg each of the reporter plasmid E2-CAT, one of pJ4Q, pJ4QRb-c or pJ4QRb-107 and 2 µg of internal control plasmid. CAT activities are expressed relative to the negative control, ± 1 S.D.
presence of pRb and p107 in the larger complexes may mask a trans-activating function encoded by E2F. First, pRb both trans-represses the E2 promoter and is present in one of the larger complexes. Secondly, adenovirus E1a trans-activates the adenovirus E2 promoter and the larger complexes are absent in E1a-expressing cells. Third, E2F has recently been shown and its over-expression shown to activate E2F-dependent transcription (Helin et al., 1992; Kaelin et al., 1992; Shirodkar et al., 1992). Fourth, detailed analysis of E2F activity indicates a cell cycle variation in complex formation: the small complex is more abundant in S phase of the cell cycle, the pRb-containing complex is present mainly in G1 and the p107-containing complex in G2. Since E2F sites are found in the promoters of many of the genes whose expression is limited predominantly to the S phase, activation by free E2F and inhibition by E2F complexed to pRb or p107 forms an attractive explanation.

These data fit well with the demonstration here that trans-activation by E7 is partly mediated by interaction with pRb, since E7 has been shown to dissociate the E2F–pRb complexes (Pagano et al., 1992). We also suggest that E7 can mediate trans-activation through interaction with p107 in an analogous fashion. However, although Pagano et al. (1992) did not analyse the p107 complex, they demonstrated that E7 dissociates the E2F–cyclin A complex much less effectively than the E2F–pRb complex and earlier work suggests that cyclin A and p107 are present in the same E2F complex (Shirodkar et al., 1992). Therefore, it is not clear whether a p107-mediated pathway for trans-activation by E7 would be relevant to E7 in transformed cell lines where the protein might be expressed at a lower level than by transient transfection. This idea is in accord with the model presented by Shirodkar et al. (1992) since dissociation of the E2F–p107 complex would hold the cell in S phase, blocking the cell cycle. However, in a productive HPV infection, E7 might act to stimulate proliferation in the basal epithelial layers but block the cell in a pseudo S phase in the suprabasal layers where HPV DNA replication occurs, this switch being regulated by an increased concentration of E7. Although the work presented in this paper clarifies the molecular aspects of trans-activation by E7, it is clear that full understanding of its function in both the proliferative viral life cycle and transformation will require analyses in other model systems which more faithfully reproduce the normal controls on E7 expression.

References


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