The E7 oncoprotein from human Papillomavirus (HPV) mediates cell transformation in part by binding to the human pRb tumor suppressor protein and E2F transcription factors, resulting in the dissociation of pRb from E2F transcription factors and the premature cell progression into the S-phase of the cell cycle. This activity is mediated by the LXCXE motif and the CR3 zinc binding domain of the E7 protein. In this study we report the x-ray crystal structure of the CR3 region of HPV E7 and a structure-based mutational analysis to investigate its mode of pRb and E2F binding and E2F displacement from pRb. The structure reveals a novel zinc-bound E7-CR3 obligate homodimer that contains two surface patches of sequence conservation. Mutation of residues within these patches reveals that one patch is required for pRb binding, whereas the other is required for E2F binding. We also show that both E7-mediated interactions are required to disrupt pRb-E2F complexes. Based on these studies we present a mechanistic model for how E7 displaces E2F from pRb. Because the CR3 region of HPV E7 has no detectable homology to other human proteins, the structure-function studies presented here provide an avenue for developing small molecule compounds that inhibit HPV-E7-mediated cell transformation.

Human papillomaviruses (HPVs) are the causative agents for more than 90% of cervical cancers (1). More than 200 HPV genotypes have been identified based on genomic differences (2), and these genotypes fall into low risk (for example types 1A, 6B, and 11) and high risk (for example, types 5, 8, 16, 18, 31, and 33) forms that are correlated with benign and malignant lesions, respectively (3) (supplemental Fig. 1). Host cell transformation by HPV is mediated by two viral oncogenes, E6 and E7 (4–6). E6 proteins contain four CXXC motifs that presumably form two zinc binding regions (3,7–9), and E6 proteins of high risk type 16 and 18 cooperate with the host cellular factor E6-AP (E6-associated protein) to target the tumor suppressor p53 to ubiquitin-mediated degradation (10,11).

E7 proteins resemble adenovirus E1A and SV40 large T antigen both in primary sequences and in transactivation and transformation properties. Based on amino acid sequence homology within E7 proteins, they can be separated into three conserved regions denoted in an analogous fashion to adenovirus E1A as CR1, CR2, and CR3 (12). The CR2 and CR3 regions of HPV E7 share sequence homology with the corresponding regions of adenovirus E1A and SV40 large T antigen, including a strictly conserved LXCXE motif that mediates high affinity binding to pRb (13,14). The CR3 region of E7 contains two CXXC motifs that are separated by 29 or 30 residues, forming a novel zinc binding domain (3), which is also present in two copies in the primary sequence of E6 (15). The HPV E7 CR3 region has been shown to mediate protein dimerization (15–17) and to mediate direct interaction with several E7-interacting proteins. In particular, the E7 CR3 region contacts the C-terminal region of pRb (18), and full-length E7 proteins are at least 100-fold more potent in pRb binding than E7 CR1/CR2 constructs. The E7 CR3 region also mediates inactivation of the cyclin-dependent kinase inhibitors p27 and p21 (19,20) and several transcription factors that apparently contribute to HPV-mediated oncogenesis, including the TATA box-binding protein (TBP), a component of the NURD histone deacetylase complex Mi2B (21,22), the acetyltransferases p300/CBP, p300/CBP-associated factor (P/CAF) (23), and the transcription factor E2F (24).

A major cellular target for HPV-E7 and other viral oncoproteins in cell transformation is the pRb tumor suppressor. pRb represents a family of closely related proteins, including p107 and p130 that bind to E2F transcription factors and block their transcriptional activation function (25–27). Phosphorylation of pRb by cyclin/cyclin-dependent kinase results in the dissociation of pRb-E2F complexes and the transcriptional activation of E2F-regulated S-phase genes (28). pRb binding by viral oncoproteins SV40 large T antigen, adenovirus E1A, and HPV E7 results in the misregulated release of E2F transcription factors and the activation of S-phase genes. pRb contains pocket domain A and B and a C-terminal domain harboring target sites for cell cycle-dependent post-translational modifications, including phosphorylation and acetylation (29). Although the A-B pocket of pRb contains a high affinity binding site for the E2F transactivation domain, the C-terminal pRb domain also contains a lower affinity binding site for other E2F regions, and the site of pRb interaction has been mapped roughly to a region that overlaps the HPV E7 CR3 binding site (18,30). The crystal structures of the A-B pocket of pRb in complex with peptides derived from either the high affinity E7 LXCXE peptide or a E2F 18-residue activation domain peptide show that, whereas the E2F peptide binds to the cleft formed by the A/B interface, the E7 peptide binds to the B domain nearly 30 Å away. These findings are consistent with in vivo and in vitro studies showing that LXCXE peptides cannot displace E2F from pRb (31,32) and iso-
thermal titration calorimetry studies showing that the two peptides bind to pRb independently (33, 34). Moreover, these studies are consistent with observations that the E7 CR3 region cooperates with the CR2 region for E2F displacement from pRb (18) and subsequent cell transformation, although the mechanism for this is not clear.

Despite the large number of biological activities that map to the CR3 region of HPV-E7 as well as its related region within HPV-E6, this domain has previously resisted detailed structural and mechanistic analysis. In this study we now report on the high resolution x-ray crystal structure of the CR3 region of E7 from genotype 1A of HPV and use structure-based mutagenesis to dissect how the protein disrupts pRb-E2F complexes to mediate cell transformation.

**MATERIALS AND METHODS**

Protein Expression and Purification for Crystallization—DNA fragments encoding residues 44–93 of native type 1A HPV E7 (HPV 1A E7-(44–93)) protein preceded by DNA encoding an MK sequence was cloned into the pRSET A vector for protein overexpression in Escherichia coli cells BL21 (DE3) (Invitrogen). Cells were grown in LB medium supplemented with 100 μg/ml ampicillin at 37 °C, and when the A605nm reached 0.5, 1 mM isopropyl-β-D-galactopyranoside and 100 μM zinc acetate were added to the media, and the cells were grown for 3 additional hours before they were harvested by centrifugation at 4000 × g for 20 min to isolate the cell pellet that was stored at −70 °C before protein purification. Frozen cell pellets were suspended in 20 mM Tris-HCl, pH 7.5, 100 mM sodium chloride, 10 μM zinc acetate, and 10 mM dithiothreitol and lysed by sonication. The cell lysates were centrifuged at 40,000 × g for 30 min, and the insoluble fraction containing the HPV 1AE7-(44–93) was solubilized in 20 mM Ches, pH 10.15, 100 mM sodium chloride, 50 μM zinc acetate, 10 mM dithiothreitol, and 6 mM guanidine-HCl denaturant. This HPV 1AE7-(44–93) denaturant solution was dialyzed against the same buffer without guanidinium-HCl overnight followed by a 4-h dialysis against a buffer containing 20 mM Tris-HCl, pH 7.5, 50 mM sodium chloride, 10 μM zinc acetate, and 10 mM dithiothreitol. The solution containing the refolded HPV 1AE7-(44–93) protein was centrifuged to remove precipitates formed during the refolding process, and the supernatant was then applied to a pre-equilibrated ion exchange Q-Sepharose column (Amersham Biosciences). The flow-through from the Q-Sepharose column, harboring the HPV 1AE7-(44–93) protein, was collected, concentrated (Millipore), and loaded onto a Superdex 200 gel filtration column (Amersham Biosciences). Peak fractions containing HPV 1AE7-(44–93) were judged to be greater than 95% pure by SDS-PAGE analysis with a typical yield of 100 mg of recombinant protein from 12 liters of growth culture. For the preparation of selenium-derivatized protein, base substitution encoding a single L76M mutation was introduced into the expression sequence into the pGex4T-1 vector (Amersham Biosciences) and transforming sequences into the pGex4T-1 vector (Amersham Biosciences) and the pSET A vector (Invitrogen), respectively. P.Gex6p1-E2F1-(243–437) was kindly provided by Dr. Steven Gamblin (MRC, Mill Hill, UK). All site-directed E7 and pRb mutations employed the QuikChange mutagenesis kit essentially as described by the manufacturer (Stratagene). GST-E7 and GST-E2F1-(243–437) fusion proteins were expressed in BL21 (DE3) CodonPlus RIL cells (Novagen), and His-pRb fusion proteins (including wild-type and mutant his-pRbABC) were generated by cloning the corresponding coding sequences into the pGex4T-1 vector (Amersham Biosciences) and the pSET A vector (Invitrogen), respectively. P.Gex6p1-E2F1-(243–437) was subject to digestion with Precission Protease (Amersham Bioscience) to produce an untagged E2F1-(243–437) protein for further studies. GST- and His-tagged proteins were purified as described by the manufacturers (Novagen and Qiagen, respectively).

GST pull-down assays were carried out by mixing 30 μg of GST-E7 fusion protein in a batch with 20 μl of glutathione-Sepharose 4B beads (Amersham Biosciences) that were pre-equilibrated with the binding buffer 1× PBS, pH 7.4, 100 mM sodium chloride, 5 μM β-mercaptoethanol, 0.1% TWEEN 20. Equivalent molar amounts of either pRb or E2F1 protein were then added to the GST-E7 equilibrated beads in a total volume of 350 μl, and the protein/bead mixture was further equilibrated at 4 °C for 1 h accompanied by gentle mixing. Protein not bound to the glutathione beads was removed by centrifugation at 500 × g for 3 min, and the beads were washed 2 times with 1 ml of binding buffer. The beads were resuspended in 30 μl of SDS-loading dye and boiled for 5 min. Samples were analyzed on 12% PAGE Duramide gels (Cambrex) followed by either Coomassie Blue staining for pRb proteins or for E2F1...
protein using Western analysis with anti-E2F1 antibody (Genetex) for E2F1-(243–437).

E2F Displacement Assays—10 μg of His-pRb-(376–928) was pre-mixed with equivalent molar amounts of non-tagged E2F1-(243–437) and added to 30 μl of pre-equilibrated nickel nitrilotriacetic acid beads in a total volume of 200 μl of binding buffer containing 1× PBS, pH 7.4, 100 mM sodium chloride, 5 μM β-mercaptoethanol, 0.1% Tween 20 for further equilibration at 4 °C for 30 min. Increasing molar amounts of GST-E7-E/D-CR3 (10-, 20-, 30-, and 50-fold excess) or untagged E7-CR2/CR3 (1-, 2-, and 5-fold excess) relative to His-pRB input was added to the beads with bound His-pRB-E2F1 complex to a final volume of 350 μl for each displacement reaction. The reaction mixtures were mixed at 4 °C for an additional 1 h. Protein not bound to the glutathione beads was removed by centrifugation at 500 × g for 3 min, and the beads were washed 2 times with 1 ml of binding buffer before analysis of the protein-bound beads on SDS-PAGE as described above.

RESULTS

Overall Structure of HPV E7-CR3—The recombinant HPV 1AE7 CR3 region (residues 44–93; Fig. 1A) was prepared from bacteria and crystallized in space group P21212. The crystal structure was determined using MAD from the two bound zinc atoms per asymmetric unit and refined to a resolution of 1.6 Å, with excellent crystallographic statistics and protein geometry (Table 1). The E7 CR3 domain assembles as a roughly globular and obligate dimer of approximate dimensions 30 Å × 20 Å × 25 Å (Fig. 1B). Each subunit of the dimer is formed by the face-to-face dyad-related packing of the two subunit CR1-CR2 faces and β-sheet interactions between β2 and β3 strands of opposing subunits.

FIGURE 1. Sequence alignment and overall structure of E7. A, sequence alignment of a selected set of HPV E7 proteins. Strictly conserved residues are shaded black, and conservative substitutions are shaded gray. The CR1 and CR2 regions of HPV E7 (not included in this structure) are indicated above the alignment, and the secondary structural elements of the CR3 region (included in this study) are indicated above the alignment. Also indicated above the sequence alignment of the CR3 region are residues in 1AE7 that make hydrogen bond (open circle) and van der Waals (closed circles) dimer interactions and residues implicated by the mutational studies reported here to make interactions with pRb (solid stars), E2F1 (solid diamonds), or residues that do not make detectable interactions with these proteins (solid triangles). B, a schematic structure of the HPV 1AE7 dimer is shown in two orthogonal views, with the two protomers of the dimer colored in blue and green, and the bound zinc atoms and their cysteine ligands colored in purple (ball) and yellow (stick), respectively.
Superposition of either the E7-CR3 monomer or dimer structure against a structure database using the Dali server (www.ebi.ac.uk/dali/index.html) identifies no structures with Z-scores over 2.8 (less than 2.0 is considered dissimilar). Analysis of the structures with Z-scores against a structure database using the Dali server (www.ebi.ac.uk/dali/index.html) identifies no structures with Z-scores over 2.8 (less than 2.0 is considered dissimilar). Analysis of the structures with Z-scores against a structure database using the Dali server (www.ebi.ac.uk/dali/index.html) identifies no structures with Z-scores over 2.8 (less than 2.0 is considered dissimilar). Analysis of the structures with Z-scores against a structure database using the Dali server (www.ebi.ac.uk/dali/index.html) identifies no structures with Z-scores over 2.8 (less than 2.0 is considered dissimilar). Analysis of the structures with Z-scores against a structure database using the Dali server (www.ebi.ac.uk/dali/index.html) identifies no structures with Z-scores over 2.8 (less than 2.0 is considered dissimilar). Analysis of the structures with Z-scores against a structure database using the Dali server (www.ebi.ac.uk/dali/index.html) identifies no structures with Z-scores over 2.8 (less than 2.0 is considered dissimilar). Analysis of the structures with Z-scores against a structure database using the Dali server (www.ebi.ac.uk/dali/index.html) identifies no structures with Z-scores over 2.8 (less than 2.0 is considered dissimilar). Analysis of the structures with Z-scores against a structure database using the Dali server (www.ebi.ac.uk/dali/index.html) identifies no structures with Z-scores over 2.8 (less than 2.0 is considered dissimilar).
of the CR2 within the HPV protein increases its pRb affinity by about 300-fold (Supplemental Fig. 2). In addition, the E/D-rich region of E7 (located between the CR2 and CR3 regions of E7) was included (called GST-E7-E/D-CR3) because this region had previously been implicated to make weak pRb interactions (43) and because we anticipated that it may serve as a flexible linker between GST and the minimal CR3 domain of E7. From these experiments, wild-type and mutant E7 proteins from genotypes 1A and 16 showed similar patterns for pRb binding (Fig. 4B). Specifically, although most mutations of residues within patch 1 and patch 2 of E7 did not affect pRb binding, the E74R/E75R double mutant within patch 1 of HPV 1AE7 and the corresponding E80R/D81R double mutant of 16E7 reduced pRb binding by ∼5- and 6-fold, respectively. This experiment suggests that the electronegative region within patch 1 of the E7 proteins mediates pRb interaction. Interestingly one of the patch 2 mutants R66E of 16E7 showed abnormal electrophoretic properties and enhanced pRb binding affinity (Fig. 4B).

Given that our studies implicate that an acidic region of E7-CR3 mediates pRb binding and that a previous study mapped CR3 binding to residues within 803–841 of the C domain of pRb (33), a region harboring several strictly conserved lysine and arginine residues, we addressed whether basic residues in this region of pRb may mediate interaction to patch 1 of E7-CR3. Three single pRb mutants K810E, K814E, K824E, a triple mutant K810E/K814E/K824E, and a quintuple mutant K810E/K814E/K824E/R828E/R830E were made and subject to GST pull-down assays with native GST-1AE7-E/D-CR3 and GST-16E7-E/D-CR3. Although none of the single lysine mutants within the C domain of pRb had significant effects on E7 CR3 binding, the pRb mutants containing multiple lysine substitutions resulted in a 3–5-fold reduction in E7 CR3 binding (Fig. 4C). This was true for both the 1A and 16 genotypes of HPV E7. Together these studies suggest that there is an electrostatic contribution to the interaction between the E7 CR3 domain and the C region of pRb.
The finding that there is an electrostatic component to the interaction between an acidic patch on E7-CR3 and a basic region within the C-domain of pRb suggested that mutations that reversed the charge in the interacting surfaces of both the E7 and pRb proteins might support binding between the two proteins. To test this hypothesis, GST pull-down experiments were carried out to compare the binding of the E74R/E75R 1AE7 and the E80R/D81R 16E7 CR3 domain mutants to native pRb as well as several charge reversal mutants within the C domain of pRb. These experiments revealed that the E7 mutants bound pRb less well than wild-type E7 (Fig. 4D), and as hypothesized, several single lysine to glutamate mutations within the C domain of pRb (K810E and K814E) restored pRb binding to the mutated E7 proteins from genotypes 1A and 16 to near wild-type levels (Fig. 4C). As a control, a single lysine mutant, K844E, outside the E7 binding region of pRb, failed to restore binding between pRb and the E7 mutant (Fig. 4D). Overall, the E7 mutants interacted less efficiently with the pRb C domain mutants containing multiple basic to acidic substitutions, implicating that the electrostatic contribution to the interaction between the pRb C domain and the E7-CR3 domain is not the sole determinant for binding between these protein domains.

Although our studies reveal an interaction between the E7 CR3 domain and the C domain of pRb in the context of a pRbABC construct, similar pull-down studies between the E7 CR3 domain and the isolated C domain of pRb (residues 803–841) in the form of an N-terminal MBP fusion protein failed to show an interaction between the two proteins (data not shown). This result demonstrates that the A-B pocket domain...
of pRb must also cooperate with the pRb C region for E7 CR3 binding. Moreover, the observation that pRb binds to two relatively divergent genotypes of E7, type 1A and type 16 (Supplemental Fig. 1), with similar sensitivities to mutation further suggests that the mode of pRb binding to E7-CR3 is conserved among E7 proteins from different HPV genotypes.

*E7 CR3-mediated disruption of pRb-E2F Complexes*—A major transforming activity of HPV correlates with the ability of the E7 protein to disrupt pRb-E2F complexes. To directly determine whether the CR3 region of E7 contributes to the disruption of pRb-E2F complexes, we prepared a 1:1 complex of bacterially expressed His-tagged pRb large pocket (His-pRbABC) and a bacterially expressed E2F fragment harboring residues 237–423 that was previously shown to be sufficient for pRb binding (33). To this pRb-E2F complex, we titrated in increasing amounts of GST-E7/E/D-CR3 domain from either genotypes HPV 1A or HPV 16. The amount to E2F retained in complex with pRb was then analyzed by Western analysis. Figs. 5, A and B, show that the E7 CR3 proteins from both HPV genotypes 1A and 16 disrupted pRb-E2F complexes in a dose-dependent manner, although these recombinant proteins were much less potent for pRb displacement than the LXCXE-containing E7 and E1A protein constructs (Supplemental Fig. 3), confirming a requirement for the LXCXE motif for full displacement. To address whether the pRb-E2F displacement activity of HPV E7 was dependent on the interaction between patch 1 of the E7 CR3 domain and the C-domain of pRb, we carried out the similar experiments using either the E74R/E75R 1AE7 or K810E/K814E/K824E pRb mutants. Figs. 5, C and D, show that neither of these mutants supports the E7-mediated displacement of pRb-E2F complexes. Similar results were observed for the E80R/D81R 16E7 mutant (Figs. 5, D and F). This data demonstrate that the interaction between patch 1 of the E7 CR3 domain and the basic region within the C domain of pRb contributes to the disruption of pRb-E2F complexes.

Because the K810E pRb mutant restored the binding of pRb to the E7 patch 1 charge reversal mutants (1AE7 E74R/E75R and 16E7 E80R/D81R), we asked whether these E7 mutants might promote E2F displacement from the K810E pRb mutant. As can be seen in Fig. 5G, the E7 charge reversal mutants are unable to disrupt preformed pRb-E2F complexes, suggesting that E7-CR3 binding to the basic region of the pRb C domain is not sufficient for disruption of pRb-E2F complexes.

Because E2F was previously reported to bind directly to the CR3 region of HPV16 E7 (24), we asked whether this interaction might be mediated through patch 1 and/or patch2 of E7-CR3 and whether this interaction contributed to E7-CR3-mediated disruption of pRb-E2F complexes. For these studies we carried out pull-down studies with E2F-(237–423) using wild-type, patch 1, and patch 2 mutants of GST-E7/E/D/CR3. The wild-type E7 protein efficiently pulls down E2F, as expected (Fig. 6A). However, patch 2 mutants, harboring R60E and L61Q substitutions (residues that are strictly conserved across most HPV genotypes), show a reduction in E2F binding by about 5-fold for each mutant. To address whether the interaction between patch 2 of E7/E/D/CR3 and E2F is important for E7-mediated disruption of pRb-E2F complexes, we assayed the ability of the patch 2 E7-CR3 mutants (R60E for 1AE7 and R66E for 16E7) to disrupt the complex. We found that the arginine to glutamate mutants of E7 patch 2 were unable to disrupt pRb-E2F complexes in either the context of 1AE7 or 16E7 (Figs. 6, B and C). Mutation of the conserved leucine residue within patch 2 (Leu-61 in 1AE7 and Leu-67 in 16E7) has been previously shown to disrupt other E7-mediated protein contacts, including loss of binding.

![Structure of the HPV E7 Oncoprotein](image_url)
to P/CAF and loss of histone deacetylase interaction (22, 23), whereas the pRb binding capacity of this E7 mutant remains unchanged. Although mutation of this residue also disrupts E2F binding (Fig. 6A) and the E2F displacement activity of E7 (data not shown), it may also destabilize the structure of the E7-CR3 dimer, since this residue sits at the interface of the homodimer. This observation may explain the detrimental nature of this mutation in E7. Together, these studies demonstrate that patch 2 of E7-CR3 mediates E2F interaction, and this interaction is required for disruption of pRb/E2F complexes.

DISCUSSION

Crystallographic studies of the pRb pocket domain bound to either a high affinity LXCXE motif within the CR2 region of HPV E7 (34) or a high affinity transactivation peptide of E2F (33, 44) have revealed that, whereas the E2F peptide binds to a groove between the A and B domains of pRb, the E7 peptide binds to the B domain nearly 30 Å away from the E2F peptide. This is consistent with the observation that the E7 peptide binds to a groove between the A and B domains of pRb, whereas the E2F peptide binds to a groove containing E7 construct (33). Putting these previous studies together with our findings that the E7 CR3 region harbors binding sites for both the C domain of pRb and the marked box region of E2F and contributes to the disruption of pRb/E2F complexes through these interactions, we are able to propose the following model for E7-mediated dissociation of pRb/E2F complexes (Fig. 7). First, E2F binds to pRb through at least two sets of interactions: that is, an interaction between the E2F transactivation domain and the pRb-A/B pocket and an interaction between the E2F-marked box with the pRb-A/B pocket and C-domain. We also propose that the interaction between the E2F marked box and the pRb C-domain involves the basic region of the pRb C domain. HPV E7 then mediates disruption of the pRb/E2F complex by first forming a stable complex with pRb via an interaction between the LXCXE-containing CR2 region of HPV E7 and the B domain of the pRb pocket region. This high affinity pRb/HPV E7 interaction then presents patch 1 of the HPV E7 CR3 domain to make low affinity contacts to the basic region within the C-domain or pRb and patch 2 of the CR3 domain of HPV E7 to make contacts to the C-terminal region marked box region of E2F. We propose that the HPV-E7 CR3 mediated destabilization of the pRb/E2F marked box interaction is sufficient to drive E2F displacement from pRb. Although we do not currently know the mechanism for destabilization of the interaction between the pRb-A/B pocket and the E2F transactivation domain, it may occur through some allosteric mechanism (Fig. 7A). Additionally, it is well established that cyclin-dependent kinase/cyclin phosphorylation of the pRb-C domain leads to the displacement of E2F and, specifically, to the displacement of the transactivation domain of E2F (28, 33, 45). In light of this, it is also possible that displacement of the E2F marked box from the pRb C domain makes the C domain more accessible to cyclin-dependent kinase/cyclin-mediated phosphorylation in vivo, which in turn leads to the displacement of the E2F transactivation domain from the pRb pocket region (Fig. 7B). Consistent with this hypothesis, the adenovirus E1A also has been shown to modulate the phosphorylation levels of the pRb pocket proteins (46). Interestingly, the cyclin-dependent kinase/cyclin phosphorylation sites within the C domain of pRb are located within the basic region of the pRb C-domain that mediates HPV E7-CR3 binding.

In this study we have determined the high resolution crystal structure of the CR3 domain of the HPV E7 protein and have employed structure-based mutagenesis to address the molecular basis for how this domain of HPV E7 perturbs the function of the human pRb tumor suppressor protein. In particular, we have identified two conserved surface patches on the E7 CR3 domain that mediate its pRb inhibitory function. High risk forms of HPV are causative agents for cervical cancer and are, therefore, important therapeutic viral targets. In light of the fact that the HPV E7 protein plays a key role in cell transformation and our observation that the E7 CR3 domain contains a novel protein fold with no structural homologues in humans, we propose that the CR3 domain of E7 may be an excellent candidate for targeted inactivation by small molecule compounds. In particular, the development of molecules that bind patch 1 and/or patch 2 of the E7 CR3 domain may disrupt the ability of E7 to perturb pRb function. In addition, the CR3 domain of E7 has been implicated in mediating inactivation of several other human proteins associated with human cancer including histone deacetylases (22) and the p300/CBP acetyltransferases (47, 48). Therefore, small molecule compounds that specifically bind to the HPV-E7 CR3 domain might disrupt other cell-transforming activities of this virus. Indeed, a recent report has demonstrated that the integrity of the CR3 domain of HPV-E7 is essential for the life cycle of HPV (49). The structure-based development of small molecule HPV-E7 CR3-inactivating compounds may, therefore, be a therapeutically beneficial avenue of investigation.

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