COMMENTARY

Mechanisms by which DNA tumor virus oncoproteins target the Rb family of pocket proteins

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Small DNA tumor viruses have evolved different mechanisms to abrogate the function of the retinoblastoma tumor suppressor (pRb). Studies of these viruses have been invaluable in uncovering the central role of the Rb family of pocket proteins in cell cycle control. While the molecular mechanisms by which the viral oncoproteins inactivate the Rb family are still being elucidated, it is clear that targeting of this family is required both for viral replication and for virus-induced transformation of mammmalian cells. This review compares and contrasts the approaches DNA tumor viruses have evolved to antagonize Rb family members—ranging from relatively simple equilibrium dissociation of pRb from cellular pRb-binding factors to chaperone-mediated alterations in pocket protein stability and phosphorylation levels. The review will focus on the viral oncoproteins adenovirus E1A, human papillomavirus E7 and the large T antigens of several polyomaviruses. An understanding of these mechanisms may provide further insight into the regulation and functions of Rb family members as well as uncover new targets for the development of novel anti-viral agents, particularly against human papillomavirus, which is a significant cause of human cancer.

Introduction

Why do small DNA tumor viruses target the Rb family? Adenoviruses, human papillomaviruses and polyomaviruses such as SV40 rely on the host cell DNA replication machinery for replication of viral genomes. Although the host DNA replication machinery becomes available during S-phase, the viruses replicate in differentiated and, thus, non-dividing cells. Therefore, productive infection depends upon the ability of the virus to induce S-phase entry of the host cell in the presence of anti-proliferative signals. Signals associated with differentiation, growth factor deprivation, contact inhibition, DNA damage and other arrest-inducing conditions feed into Rb pathway, resulting in G1 arrest and, in some cases, exit from the cell cycle into G0 (quiescence).

The Rb family of so-called pocket proteins (Figure 1) negatively regulates progression from G0 through to G1 and into S-phase. The reader is referred to several excellent reviews for more in depth discussion of the individual Rb family members (1–4). The term ‘pocket protein’ derives from the conserved binding pocket through which pRb, p107 and p130 bind viral oncoproteins and cellular factors such as the E2F family of transcription factors. E2F family members hetero-dimerize with a binding partner (DP) to form a functional transcriptional regulator of genes involved in cell cycle progression, DNA synthesis, apoptosis and other cellular processes. These genes include, but are not limited to, cyclin E, cyclin A, B-Myc, ribonucleotide reductase, DNA polymerase α, thymidine kinase and proliferating cell nuclear antigen (PCNA) (5–7). pRb interacts with E2F1, E2F2 and E2F3. These E2F family members are generally thought of as transcriptional activators because they activate transcription when not bound by repressor molecules such as pRb (2). In addition to binding to the transcriptional activation domain of E2Fs 1–3 (8), pRb recruits chromatin remodeling factors such as histone deacetylase 1 (HDAC1) (9–12) and Brg1/Brm1 (13,14) to actively repress E2F-dependent transcription. p107 and p130 interact with E2F4 and E2F5, which are weak transcriptional activators when not bound by Rb family members (4), p107 and p130 also recruit HDACs to E2F-controlled promoters (12). For the purpose of this commentary, the various E2F family members and their dimerization partners will be referred to simply as ‘E2F’.

The expression patterns of Rb family members differ during the G0/G1/S-phase transition, with p107 highly expressed during G0, p107 highly expressed in S-phase and pRb expressed at a fairly steady level throughout the cell cycle (4). This observation, together with the fact that different pocket proteins bind to distinct E2F family members, suggests the pocket proteins may regulate various E2F target genes at different times during the G0/G1/S-phase transition (4). Significant, introduction of either pRb, p107 or p130 into certain cell lines results in G1 arrest, implying that these proteins share overlapping functions as well (15,16). Moreover, mouse embryo fibroblasts lacking all three pocket proteins lose the ability to arrest in G1 in response to a variety of anti-proliferative signals (17,18). The pocket proteins are regulated in part via phosphorylation by cyclin-dependent kinases (CDKs) including cyclin D/CDK4 (or CDK6), cyclin E/CDK2 and cyclin A/CDK2 (2). The regulation of pRb by phosphorylation is the best understood. Hyperphosphorylation of pRb results in a loss of binding to both E2F and to chromatin remodeling factors, and reverses pRb-mediated cell cycle arrest by pRb (2,9,12). Similar to pRb, p107 and p130 regulate cell cycle progression via interactions with E2F and are CDK substrates (4). However, in contrast to pRb, they also act as CDK inhibitors, which appears to contribute to their function in controlling cell cycle progression (19–21).

Inactivation of Rb family members by E1A, E7 and polyomavirus large T antigens leads to increased E2F activity and possibly to derepression of other factors inhibited by pocket proteins. Interestingly, only one of the viruses, adenovirus, depends on E2F for expression of viral genes while HPV and polyomaviruses do not (22). Therefore, inactivation of the Rb

Abbreviations: CR, conserved regions; CDK, cyclin-dependent kinases; HDAC1, histone deacetylase 1.
family by E1A, E7 and polyomavirus large T antigens may serve mainly to induce transcription of E2F-controlled cell cycle and DNA synthesis genes, thereby establishing an environment permissive for viral replication (22). Furthermore, the same regions of E1A, E7 and large T antigens required for targeting Rb family members are also required for the transforming activity of these oncoproteins (discussed below).

**Inactivation of the Rb family by adenovirus E1A**

Adenovirus 12 was the first human virus demonstrated to induce tumors in an animal model (23). Although adenoviruses have not been associated with human tumors, a number of adenovirus serotypes, such as types 2 and 5, transform tissue culture cells while other types are also oncogenic in rodent models (24). The adenovirus E1A oncoprotein is encoded by several alternatively spliced mRNAs, with the predominant forms being the 12S and 13S mRNAs (24). The 13S mRNA product contains a zinc finger not present in the 12S mRNA product (24). Both forms of E1A target the Rb family and have transforming activity and will not be distinguished from one another here. Two regions of conserved sequence among E1A proteins of different adenovirus types are conserved regions 1 and 2 (CR1 and CR2) (Figure 2A). CR1 and CR2 contribute to E1A-induced cell cycle progression and transformation (25–33).

E1A was found to bind a 105–110 kDa cellular phosphoprotein later identified as pRb (27,34–36). This important finding triggered widespread interest in the biochemical and functional interactions of viral oncoproteins with cellular tumor suppressor genes. In fact, both p107 (35–38) and p130 (35,39–43) were discovered by their association with E1A. E1A binds to the pocket domain of pRb (Figure 1), a region mutated in many cancers (44–46). E1A binds to a similar region of p107 and p130 (42,47). Within E1A CR2 is an ‘LxCxE’ motif that is necessary for high affinity binding to Rb family members (27,30,39,48–50) and is present in a number of cellular pRb-binding proteins, including HDAC1 (51). CR1 also binds Rb family members, but with lower affinity than CR2 (48,50,52).

**Disruption of pocket protein/E2F complexes**

How does the binding of E1A to pocket proteins lead to their inactivation? A number of studies have shown that E1A

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**Fig. 1.** The Rb family of pocket proteins. The small pocket region is composed of two structural domains ‘A’ and ‘B’ that are linked by a spacer region, which is larger in p107 and p130. The large pocket contains the small pocket plus the C-terminus. Viral oncoproteins bind to the small pocket while the large pocket is essential for E2F binding. p107 and p130 differ from pRb in the presence of cyclin-binding sites within the spacer region and the N-terminus. These sites bind cyclin E/CDK2 and cyclin A/CDK2. p107 and p130 also contain sequence in the B domain that is not found in the B domain of pRb. pRb, p107 and p130 also differ with regard to binding to E2F family members, with pRb binding to E2F1, E2F2 and E2F3 and p107/p130-binding E2F4 and E2F5.

**Fig. 2.** Regions of adenovirus E1A, HPV E7 and polyomavirus large T antigens important for targeting the Rb family. For information on other binding partners/activities of these tumor virus oncoproteins, the reader is referred to Saenz-Robles et al. (2001), Gallimore and Turnell (2001), Munger et al. (2001), Ali and DeCaprio (2001), Brodsky and Pipas (1998). (A) E1A. The product of the 12S mRNA is depicted here. CR1 and CR2 are boxed in gray. The LxCxE motif important for pocket protein binding lies within CR2 (thick arrow). CR1 contains a lower affinity-binding site for Rb family members (thin arrow). E1A recruits the acetyltransferase p300 into complex with pocket proteins (see text for details). (B) E7. CR1 and CR2 are indicated. As with E1A, the LxCxE motif lies within CR2. The C-terminal zinc finger (‘ZF’) binds pRb with low affinity and does not share sequence homology with other viral oncoproteins. (C) Large T antigens of SV40, JCV and BKV (SVLgT, JCLgT and BKLgT, respectively). All three T antigens contain J domains (boxed in black) that recruit the cellular chaperone HSC70, and possess LxCxE motifs within CR2. The portion of SVLgT C-terminal to CR2 is not essential for binding to Rb family members; however, there are data suggesting that the C-terminus contributes to the interaction with p107 and p130. SVLgT, JCLgT and BKLgT also have homology to E1A and E7 CR1.
E1A from complex with pocket proteins.

C) p130 that overlaps with the E2F-binding site (only pRb is depicted). This allows for CR1 to contact a site on pRb/p107/Rb family member via the LxCxE motif in CR2. This

E1A has been proposed (Figure 3) wherein E1A

CR1 and CR2 are involved (53,54,56,64,70). These studies demonstrated that CR2 and p300 is necessary for E1A to induce an entire cell cycle (28,33,49,83). Perhaps E1A recruits pRb and p300 into a complex to either inhibit or stimulate transcription of cellular genes beyond E2F during adenovirus infection (24).

Inactivation of the Rb family by HPV 16 E7

In contrast to adenoviruses, HPVs are known etiologic agents of human cancers. That HPVs induce cervical cancer was proposed in the 1970s (84) and became clear in the early 1980s when DNA from HPV types 16 and 18 were found in cervical cancers (85,86), and subsequently by studies showing that most, if not all, cervical cancers are associated with HPV (reviewed in ref. 87). Indeed, prior infection with HPV is linked with a number of anogenital malignancies (87). While HPV-associated oncogenesis is linked at least in part to the dependence of viral replication on host cell proliferation, productive infection is not observed in HPV-positive tumors, which generally arise years after the initial infection (87).
However, the HPV oncogenes E6 and E7 are always expressed in HPV-positive tumors, are sufficient for immortalization of primary human cells (87) and are required for maintaining the transformed phenotype of HPV-positive tumor cells (88–90).

Expression of E7 alone is sufficient for transformation of established rodent cell lines and for bypassing cell cycle arrest associated with, for example, differentiation, DNA damage, contact inhibition or serum deprivation. Three regions of E7 are required for these various activities (48,91–95). In the N-terminal half of E7 are CR1 and CR2 that share sequence homology with E1A CR1 and CR2 (Figure 2) (96). The third region is an unusually large zinc finger in the C-terminal half of E7 which does not share homology with E1A (91,94,97–99). Like E1A, E7 possesses an LxCxE motif in CR2. The LxCxE motif and, hence, binding to Rb family members is essential for E7 to bypass cell cycle arrest. E7 binds the G0/G1-specific, hypophosphorylated form of pRb (100,101).

Disruption of pocket protein/E2F complexes

The first mechanism proposed for how E7 functionally antagonizes pRb was by disruption of pRb/E2F complexes (67,102). pRb/E2F complexes are not apparent in HPV-positive tumor cells or in cells expressing E7 alone (67,102). Furthermore, E7 blocks pRb/E2F binding in vitro (102,103), dependent upon the integrity of the LxCxE motif (102). Both CR1 (104) and the C-terminus (62,103) of E7 function in blocking pRb/E2F binding. The E7 C-terminus has been proposed to have a low affinity interaction with a site on pRb that overlaps with the E2F-binding site (105). In contrast to E1A CR1, E7 CR1 has not been shown to bind pRb. However, when anchored to pRb via the LxCxE motif in CR2, E7 CR1 can inhibit pRb/E2F binding, suggesting that CR1 may sterically hinder access of E2F to its binding site on pRb (104). Interestingly, E7 can disrupt the p107/E2F complexes in G0/G1 (70), but not the S-phase-specific p107/E2F complex (67,69,70). This is in contrast to E1A, which disrupts both the G0/G1 and S-phase complexes, although why adenovirus targets the S-phase complex and HPV does not is unknown (69,70).

Degradation of Rb family members

A more recent proposal for how E7 inactivates pRb, that is not mutually exclusive with dissociation of pRb/E2F complexes, is through the targeted destruction of pRb. Expression of E7 in a variety of cell types results in reduced levels of pRb (106–110). The reduction of pRb is due to a loss of protein stability (107,109,110) and is dependent upon the integrity of the E7 LxCxE motif (108–110). A dramatic reduction in the level of pRb occurs rapidly after E7 expression in cells, suggesting that pRb destabilization is a direct effect of E7 (110). Interestingly, E7 CR1 is also important for destabilizing pRb, although this region is dispensable for binding to pRb (104,108–112). In contrast, the C-terminus of E7 appears not to have a direct role in targeting pRb for degradation (104,112). p107 and p130 are also targeted for degradation by E7 (104,112), and degradation of the Rb family is proteasome-dependent (107,110,112).

A mutation in CR1, H2P (histidine number 2 changed to proline) separates the ability of E7 to block pRb/E2F binding and to reduce pRb half-life (104). This CR1 mutation also prevents E7 from efficiently bypassing pRb-imposed arrest of SAOS2 cells, suggesting that the dominant mechanism by which E7 inactivates pRb is via targeted destruction of pRb (104,112). These observations do not eliminate a role disruption of pRb/E2F complexes by E7, and it is possible that E7 must first isolate pRb, p107 and p130 from complex with E2F (or other factors) before targeting them for degradation (Figure 4). This may facilitate destruction of Rb family members or, perhaps, prevent E2F from being targeted to the proteasome along with the pocket proteins.

Significantly, the E7 H2P mutant was shown to activate cyclin E- and, to a lesser extent, cyclin A-promoters in rodent cells (113,114), suggesting that the ability of E7 to block pRb/E2F binding may be sufficient for activating at least some E2F-controlled promoters. E7 is rapidly turned over (115–
Thus, during an HPV infection there may not be enough E7 to efficiently inactivate Rb family members via competition with other pocket protein-binding proteins (109,119). The ability of E7 to accelerate the destruction of pocket proteins may allow small amounts of E7 to efficiently prevent the interaction of pRb/p107/p130 with E2F and/or other cellular factors. Interestingly, the observation that E1A blocks pRb/E2F binding more efficiently than does E7 (62) suggests that E1A may not need to reduce expression levels of the Rb family members to efficiently bypass their inhibition of cell cycle progression. However, the turnover rate of E7 and E1A are similar (115,116,118,120) suggesting that their different approaches to inactivating the Rb family may not be linked to differences in their expression levels.

The reason why the integrity of CR1 is important for E7 to destabilize Rb family members is not clear. It is tempting to speculate that this region of E7 recruits cellular factors to facilitate pocket protein degradation. E7 was reported previously to bind the S4 subunit of the 26S proteasome, but this interaction was not perturbed by CR1 mutations (110). Rather, the S4 interaction was blocked by a mutation in one of the zinc-binding motifs in the C-terminus of E7 (110). Furthermore, this same mutation did not prevent E7 from destabilizing pRb, indicating that S4-binding may be important for E7 activities other than pocket protein destabilization (112).

**SV40 large T antigen**

Following its isolation from monkey kidney cells over 30 years ago (121), SV40 was found to be oncogenic upon injection into baby hamsters (122,123) and to transform rodent cells and other cells where productive viral infection does not occur (see, for example refs 124,125). Although the natural host of SV40 is the Rhesus macaque, SV40 tumor (T) antigen sequences and protein have been detected in human cancers (see, for instance refs 126–128). A causal role for SV40 in human cancers is currently under debate (130).

Required for both the virus life cycle and transformation is the large T antigen of SV40 (SVLgT) (Figure 2) (131). SVLgT shares sequence homology with CR2 and CR1 of E1A and E7 (44,132–134) and binds pRb, p107 and p130 through an LxCxE motif in CR2 and recruits the HSC70 co-chaperone via the J domain (158). The precise mechanism by which E7 and E1A alter p107 and p130 phosphorylation appears not to affect their ability to inhibit CDKs (19,20). Interestingly, SVLgT also induces proteolysis of p130 (129,158), but not pRb (129,137,140). In the presence of SVLgT, both endogenous p107 and p130 or exogenously added p130 became hypophosphorylated (129). The effect of SVLgT on p107 and p130 phosphorylation is direct, as it requires pocket protein binding through the LxCxE motif (129). How SVLgT alters the level of p107 and p130 phosphorylation is unknown, although the requirement for an intact J domain (129,158) suggests a chaperone-mediated conformational change of p107 and p130 interferes with their phosphorylation by CDKs (163). Although the ability of SVLgT to alter p107 and p130 phosphorylation correlates with a bypass of p107/p130-imposed cell cycle arrest, it is not clear how this occurs. SVLgT could perturb the ability of p107 and p130 to act as CDK inhibitors (19–21) by altering their own phosphorylation state. Accordingly, pRb does not act as a CDK inhibitor (19,20) and its level of phosphorylation is not affected by SVLgT (137). However, the level of p107 and p130 phosphorylation appears not to affect their ability to inhibit CDKs (19,20).

Interestingly, SVLgT also induces proteolysis of p130 (Figure 5D), but not the other Rb family members (129,158). Again, both the LxCxE motif and the J domain are required for this activity (158). The precise mechanism by which degradation of p130 is induced is not clear, although it is
tempting to speculate that chaperone-mediated unfolding of p130 facilitates its degradation. Alternatively, modulation of p130 phosphorylation by T antigen may lead to degradation of p130 (158). However, SVLgT alters the phosphorylation of p107 (129,158) without destabilizing p107 (129,158). Moreover, it is the most highly phosphorylated species of p130, rather than the hypophosphorylated form, that is unstable (3). p107 and p130 are highly related, with ~50% sequence identity (4), thus it is interesting that SVLgT targets p130, but not p107, for proteolysis. Perhaps these observations point to non-redundant roles for p107 and p130 in regulating cell proliferation that SVLgT must overcome to allow SV40 replication.

J domain-independent targeting of pocket proteins

Despite the necessity for the J domain of SVLgT to disrupt pocket protein/E2F complexes (147,164), to alter p107 and p130 phosphorylation levels and to induce proteolysis of p130 (129,158), there is evidence that J domain-independent functions also contribute to pocket protein inactivation. For instance, using reporter assays, Chao et al. (165) showed that SVLgT could induce a promoter containing 3 E2F sites in a J domain-independent, but LxCxE-dependent, manner (165). Indeed, the authors suggest that the J domain-independent activation of the E2F-dependent promoter could have been due to dissociation of complexes of pRb with transcriptional repressors such as HDAC1 that interact with the LxCxE-binding site of pRb (165). Moreover, another study showed that, in an assay of cell cycle arrest, an SVLgT LxCxE mutant could be complemented with an N-terminal fragment of SVLgT containing an intact LxCxE motif and a non-functional J domain (148). These studies suggest that the interaction between SVLgT and pocket proteins has other, yet to be determined consequences on pocket protein activities.

BKV and JCV T antigens

BKV and JCV are highly related to SV40 and were isolated in the early 1970s (166,167). With a prevalence of ~80% worldwide, both BKV and JCV are common viruses in the human population, where the viruses normally establish a persistent infection (168). In immunocompromised hosts, JCV can result in the neurodegenerative disease progressive multifocal leukoencephalopathy (167), while BKV has been associated with hemorrhagic cystitis (166,169). As with the other DNA tumor viruses, JCV and BKV are tropic for cells that are normally non-dividing and, thus, must induce S-phase to usurp host cell replication factors. Both viruses have transforming activity in tissue culture, albeit reduced in comparison with SV40, and both viruses are oncogenic in rodents (170). BKV and JCV sequences, and JCV proteins as well, have been detected in some human cancers (170). However, the link between these viruses and human oncogenesis is still under debate.

BKV and JCV each encode large T antigens (BKLgT and JCLgT) (Figure 2) that are necessary for viral replication and for transformation. BKLgT, JCLgT and SVLgT share 70–75% amino acid sequence homology (171). Both BKLgT and JCLgT contain a J domain and an LxCxE motif (132, 153,154,156,172,173) and bind to pRb, p107 and p130 (136,172,174–176). Much less is known regarding the interactions between BKLgT and JCLgT and the Rb family than is known about SVLgT-pocket protein interactions. In addition, the binding of BKLgT to Rb family members is difficult to detect due to low expression levels of the viral protein (175,177). However, BKLgT/pocket protein binding is detectable upon increased expression of BKLgT (175,177).

Disruption of pocket protein/E2F complexes

In electrophoretic mobility shift assays of pocket protein/E2F complexes, more ‘free’ (unbound by pocket proteins) E2F was observed in lysates containing BKLgT, and this increase in free E2F was dependent upon the LxCxE motif of BKLgT (177). Mutations in the J domain prevented the induction of free E2F (177). This increase in free E2F was paralleled by an increase in E2F-dependent transcription in a reporter assay (177). Remarkably, levels of free, transcriptionally active E2F were similar in cells expressing BKLgT and SVLgT despite a dramatic difference in the expression levels of the two viral proteins (177). Because of the low expression levels of BKLgT, it was suggested that the free E2F likely did not result from disruption of pocket protein/E2F complexes (177); however, it remains possible that chaperone-mediated changes in pocket protein folding induced by BKLgT could prevent re-association of Rb family members with E2F, thereby allowing a low level of BKLgT to disrupt enough of these complexes to activate E2F-dependent transcription and subsequent cell cycle progression. Alternatively, the increased E2F activity in BKLgT-expressing cells may result from a different means of targeting the pocket proteins, such as destabilization (see below) or by as of yet undiscovered mechanisms (175,177).

The mechanisms by which JCV targets the pocket protein family have not been well studied. JCLgT has a functional J domain (152,156) and thus may inactivate the Rb family in a chaperone-dependent manner similar to SVLgT (158,164). In fact, there is evidence that JCLgT may dissociate pocket protein/E2F complexes in a manner similar to SVLgT; the JCLgT J domain could functionally substitute for the SVLgT J domain in disrupting p130/E2F complexes (152).

Post-translational modification of pocket proteins

Based on the few studies of BKLgT- and JCLgT-pocket protein interactions, it appears that BKLgT, JCLgT and SVLgT have quite different effects upon the phosphorylation and levels of Rb family members. Despite its low expression levels, BKLgT was reported to reduce pRb, p107 and p130 steady state levels and what remained were the less phosphorylated species (175,177). Although it was not directly shown whether the effects on pocket protein levels and phosphorylation required the LxCxE motif or J domain (175,177), results with SVLgT (129,158) suggest a role for these regions of BKLgT. There is evidence that JCV may alter the phosphorylation and expression levels of pocket proteins. In JCV transformed cells, p130 levels were reduced and what remained was of faster mobility than that in control cells (178). In contrast, steady state levels of both pRb and p107 appeared to be increased in JCV transformed cells, and most of the pRb population was hyperphosphorylated (178).

A role for spliced (T) antigens

Interestingly, JCV encodes short, alternatively spliced T antigens (T’ antigens) that are expressed during lytic infections and in JCV transformed cells (179,180). The alternatively spliced JCV products are 135, 136 and 165 amino acids long (J’T135, J’T136 and J’T165, respectively) and contain both a J domain and an LxCxE motif (178,180). All three T’ antigens bind Rb family members, although with different affinities (178). In general, most of the cellular p107 and p130
was bound by the T' antigens, as well as by JCLgT, while only a fraction of the hypophosphorylated pRb present in the cell lysates was bound (178). The authors speculate that the T' antigens may significantly contribute to JCV-mediated inactivation of pocket proteins and suggest a similar scenario for BKV (178), which also produces alternatively spliced, ‘truncated’ versions of T antigen (179). The authors further speculate that increased E2F activity reported in BKLgT-expressing cells (177) could be due in part to BKV T' antigens that may bind and inactivate pocket proteins (178).

**Perspectives**

Much remains to be learned regarding the mechanisms by which DNA tumor viruses interfere with pocket protein function. Further dissection of viral oncoprotein/pocket protein interactions, together with powerful genetic and reverse-genetic tools like knock out mice and RNA interference, should provide more insight into the biochemical/cellular functions of pRb, p107 and p130, which are far from being completely understood. That each of the viral oncoproteins discussed here target all of the Rb family members could be interpreted to mean the pocket proteins have some redundant functions that the viruses must eliminate, for instance the imposition of G1 arrest. However, that viral oncoproteins such as SVLgT have differential effects on pRb, p107 and p130 supports the idea that each pocket protein has a unique role in regulating cellular processes that influences DNA tumor virus replication.

Interesting questions to pursue include how the viral oncoproteins alter the post-translational regulation of pocket proteins. For example, how does SVLgT-associated chaperone activity effect changes in p130 stability and in p107 and p130 phosphorylation levels? What is the role of E7 CR1 in inducing proteolysis of pRb, p107 and p130? Does E7 CR1 recruit cellular factors involved in proteolysis, similar to the recruitment of an E3 ubiquitin ligase by the HPV E6 oncoprotein (for targeting p53 for degradation)? If so, why does E1A not target Rb family members for proteolysis when it shares (for targeting pRb for degradation)? If so, why does E1A not promote JCV to more efficiently target specific Rb family members during different phases of the cell cycle or in different cell types?

Uncovering the answers to these questions will provide further insight into DNA tumor virus replication and may provide novel targets for therapeutic intervention in the case of the viruses which are pathogenic to humans.

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