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Roseoloviruses manipulate host cell cycle

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During lytic infections HHV-6A and HHV-6B disrupt E2F1–Rb complexes by Rb degradation, releasing E2F1 and driving the infected cells toward the S-phase. Whereas upon infection E2F1 and its cofactor DP1 were up-regulated, additional E2F responsive genes were expressed differentially in various cells. E2F binding sites were identified in promoters of several HHV-6 genes, including the U27 and U79 associated with viral DNA replication, revealing high dependence on the binding site and the effect of the E2F1 transcription factor. Viral genes regulation by E2F1 can synchronize viral replication with the optimal cell cycle phase, enabling utilization of host resources for successful viral replication. Furthermore, it was found that infection by roseoloviruses leads to cell cycle arrest, mostly in the G2/M-phase.

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Introduction

The mammalian cell cycle is a highly regulated process. At the G1 phase cells undergo a critical check point, ensuring their readiness for DNA synthesis. This is followed by the S-phase during which the cellular genome is duplicated. Following DNA duplication, the cell progresses into the G2 phase, preparing for mitosis. Members of the E2F family serve as transcriptional activators of genes that play significant roles in cell cycle control, including: DNA replication, mitosis, the mitotic checkpoint, DNA-damage checkpoints, DNA repair, differentiation, development and apoptosis [1]. In responsive genes containing the E2F binding sequence transcription begins by binding the E2F and DP heterodimers to the E2F binding site. The E2F-DP transcription complexes are negatively regulated by members of the retinoblastoma (Rb) protein family, which block the E2F activation domain, preventing the transcription of E2F responsive genes.

The Rb protein is regulated by phosphorylation and degradation. Hypophosphorylated Rb binds E2F1 with a high affinity, leading to inhibition of E2F1 transcription activity. In the G1 phase the Rb protein is inactivated following its phosphorylation by cyclin D/CDK-4/6 and cyclin E/CDK-2 complexes, resulting in its dissociation from E2F1 and cellular entry into the S-phase [2,3].

DNA viruses synthesize significant quantities of nucleic acid during the productive lytic replication. Therefore they have evolved ways to modulate the Rb–E2F pathway. Viral inactivation of the Rb family members enables them to create an environment more accommodating for viral replication. The disassembly of Rb/E2F1 complexes by viral proteins leads to accumulation of free E2F1 transcription factor and induction of S phase entrance. Such viral proteins include the extensively studied human papillomavirus oncoprotein E7, the adenovirus E1A protein, and the SV40 large T antigen. These three viral proteins represent two distinct mechanisms of Rb inactivation: steric disruption of Rb–E2F complexes and Rb degradation [4••].

Herpesviruses encode proteins that use these pathways and additional direct and indirect mechanisms to inhibit Rb family member function.

Cells infected by the ***alphaherpesviruses*** HSV-1, HSV-2, and VZV, accumulate in the G1 phase of the cell cycle [5,6]. Moreover, while the Rb proteins remain unphosphorylated in HSV-infected cells, the activity of kinases responsible for their phosphorylation, the Cdks, is critical for HSV-1 replication [7]. Thus, the *alphaherpesviruses* may be less dependent on cellular E2F-responsive genes for viral DNA replication than other herpesviruses and may not need to target Rb family members for inactivation.

The ***gammaherpesviruses*** are associated with proliferative disorders including a number of cancers. Both EBV and KSHV appear to encode proteins that modulate the Rb–E2F pathway, either directly or indirectly. EBV has multiple proteins (Z, R, LMP-1, EBNA-2,-3C,-5), that could lead to the phosphorylation of Rb by cellular CDKs, and/or may directly phosphorylate Rb through the function of the viral kinase, BGLF-4 (ortholog of HCMV UL97). Moreover, EBV at the lytic infection, but not during latency, was shown to induce cell cycle arrest at

the G1/S with elevated levels of cyclin E and cyclin A [8]. In the KSHV infection E2F1 pathway is activated by LANA-mediated disruption of E2F1/Rb complexes, or by direct phosphorylation of Rb through the action of the v-cyclin and/or the ORF36 proteins [9,10].

Beta-herpesviruses manipulate E2F–Rb pathway during lytic infection

For the HCMV: It was demonstrated that at very early stages of infection hypophosphorylated Rb protein was first degraded, and protein synthesized de novo was then hyperphosphorylated [11]. The HCMV pp71 protein is a prominent component of the viral tegument [12,13]. By binding to Rb protein, pp71 induces Rb degradation in a proteasome-dependent, ubiquitin-independent manner [14]. HCMV UL97 has been shown to phosphorylate multiple residues of Rb, disrupting the E2F1/Rb and Rb/HDAC complexes, rendering Rb inactive. Due to both pp71-mediated degradation and UL97-mediated phosphorylation, Rb is inactivated and E2F responsive genes are highly expressed [15].

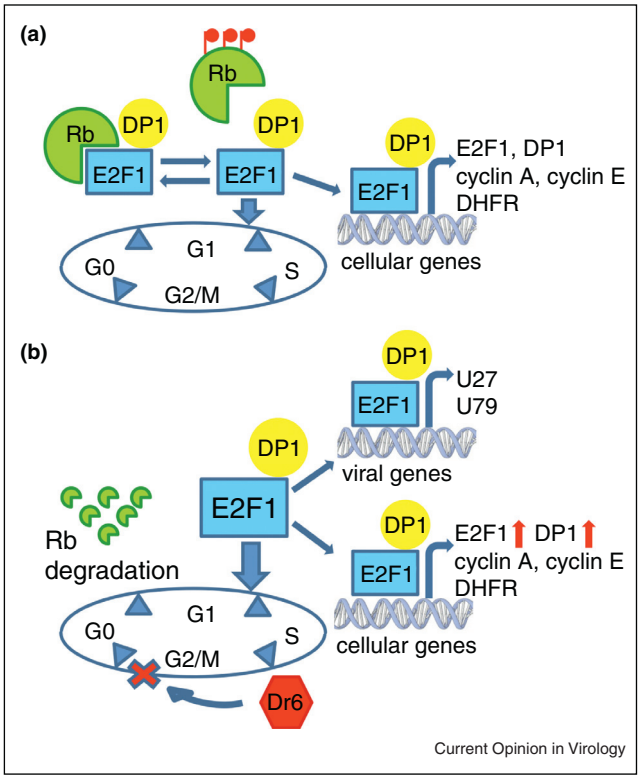
For the Roseoloviruses: We have shown [16•,17••] that in SupT1 T cells infected with HHV-6A the E2F1 protein and its co-factor DP1 increased whereas the Rb protein underwent massive degradation without hyperphosphorylation at 3 sites, Ser-780, Ser-807 and Thr-821, known to control E2F/Rb association (Figure 1). The degradation of Rb started simultaneously with E2F1 and DP1 up-regulation. Furthermore, it correlated with the accumulation of the viral p41 protein that functions in viral DNA replication. Increased E2F1 expression was also described employing a microarray assay of HHV-6B infected adult T-cell leukemia cell line [18]. Because HHV-6A infection induced elevation of free E2F1, it was of interest to monitor the expression of the E2F1 target genes and alterations of cell cycle during the infection. Although E2F1 and DP1 levels were elevated we found that cyclin A, cyclin E, DHFR and MCM5 were not up-regulated [16•,17••]. These results differ from the results of De Bolle and coworkers, who found that late post HHV-6A infection of human cord blood mononuclear cells there was increased accumulation of cyclin A without up-regulation of cyclin E [19]. Furthermore, analysis of the regulatory proteins which are involved in the cell cycle in HSB-2 cells [20], indicated that cyclins A2, B1, E1 and MCM5 were increased in HHV-6-infected cells, but there was no difference in cyclin D1. Hence, the expression of E2F1 target genes during HHV-6A infection varies in different cells/tissues examined.

Enhanced transcription of viral genes by E2F1

As described above, HHV-6A infection induces Rb degradation, up-regulation of E2F1 and DP1. However, there was no increased expression of additional E2F1-responsive genes. It was thus of interest to test whether the virus exploited E2F1 for viral gene transcription. Scanning of the HHV-6A genome revealed several genes that contained the consensus E2F binding sequence TTTSSCGC, where S is either a G or a C upstream of the ATG start codon. This included: (i) U18, a transcriptional regulator in the IE-B/E gene class. (ii) U33, a viral tegument protein that is a critical mediator of metabolic stress. (iii) U52 gene which promotes the accumulation of late transcripts. (iv) U74 gene encoding a portion of the helicase/primase complex. (v) The U27 and the U79 genes, both functioning in viral DNA synthesis [17••]. The U27 gene encodes the P41 viral DNA polymerase processivity factor [21]. The U79-80 early gene encodes a family of nuclear proteins that were found to be essential for viral DNA replication [22].

We concentrated on the U27 and U79 genes and tested whether the E2F1 transcription factor and E2F binding site were utilized for their expression [17••]. We constructed vectors containing a GFP reporter gene driven by wild-type viral promoter or by mutant promoter that

Figure 1



Mechanisms used by HHV-6 to modulate the Rb–E2F pathway and cell cycle progression. **(a)** E2F1 regulation in uninfected cells involves Rb phosphorylation and E2F1 release, leading to transcription of cellular genes and entry to S-phase. **(b)** HHV-6 infection leads to massive Rb degradation and to E2F1 release. Active E2F1 induces up-regulation of itself and its co-factor DP1 which are utilized for transcription of cellular genes as well as viral genes (U27, U79). DR6 gene product causes cell cycle arrest in G2-phase.