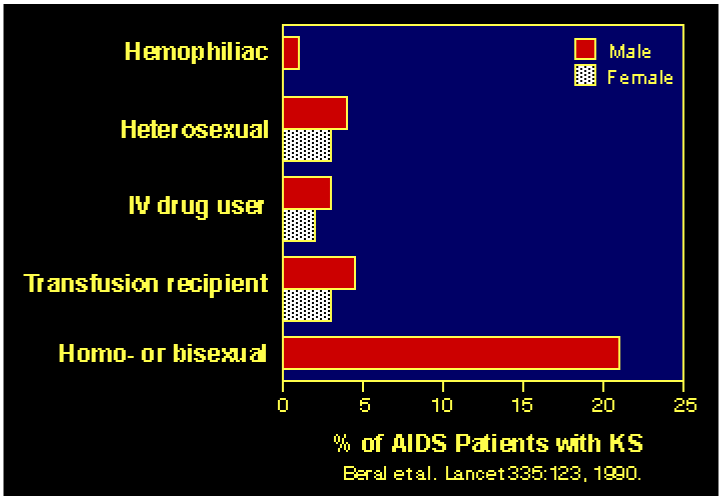
Gammaherpesviruses: HHV-8 (aka KSHV)

Neipel & Fleckenstein. Sem. Cancer Biol. 9:151, 1999
Antman & Chang. N. Engl. J. Med. 342:1027, 2000

For several years it had been thought that **Kaposi's sarcoma** (KS), an important AIDS-associated tumor, might have an infectious cause, in part because this tumor is common in HIV-infected gay men, and rather rare in other groups of HIV-infected persons.

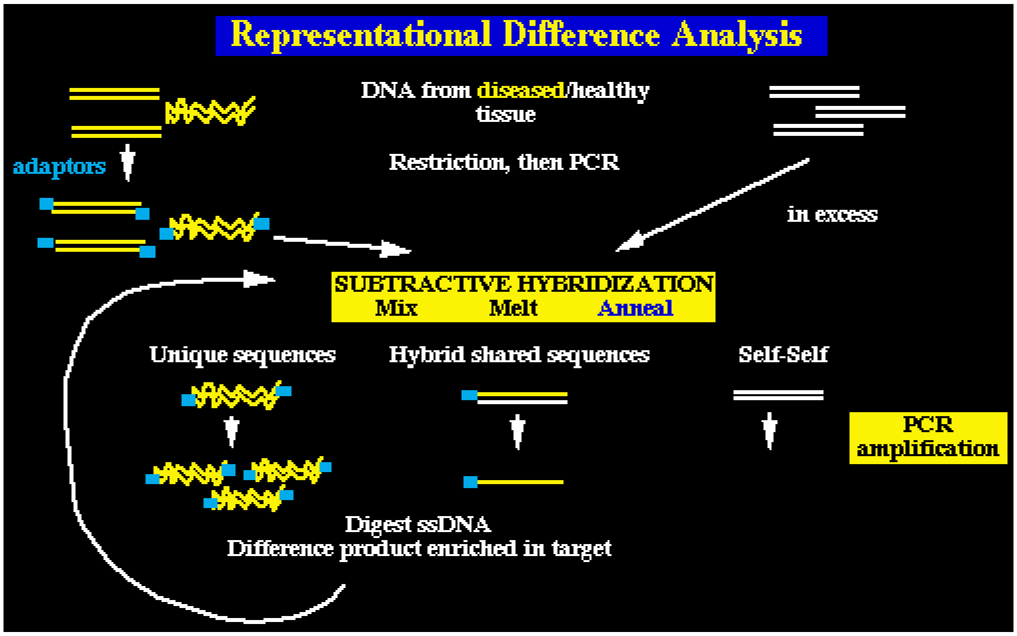
Epidemiology of KS, among persons with AIDS



Human herpesvirus 8 (HHV-8) was discovered by Yuan Chang and Patrick Moore in 1994, using KS tumor samples. Moore and Chang used a method known as **Representational Difference Analysis** (RDA), to compare DNA samples prepared from KS tumors with DNA from KS-negative persons. In the course of this analysis, they found some DNA sequences that were present *only* in the KS tissues. These turned out to be snippets of the genome of HHV-8.

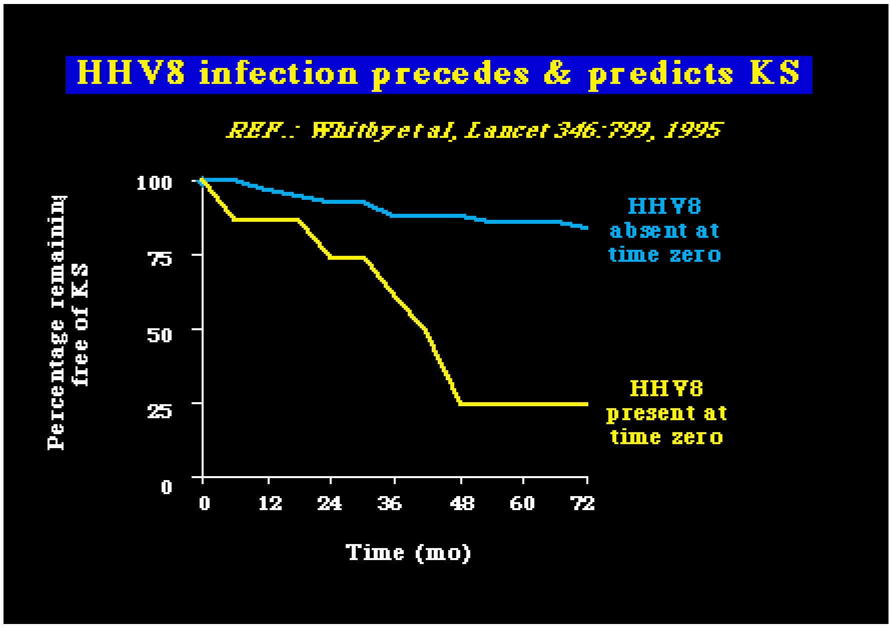
Principle of RDA analysis

Lisitsyn et al. Science 259:946, 1993; Chang et al. Science 266:1865, 1994

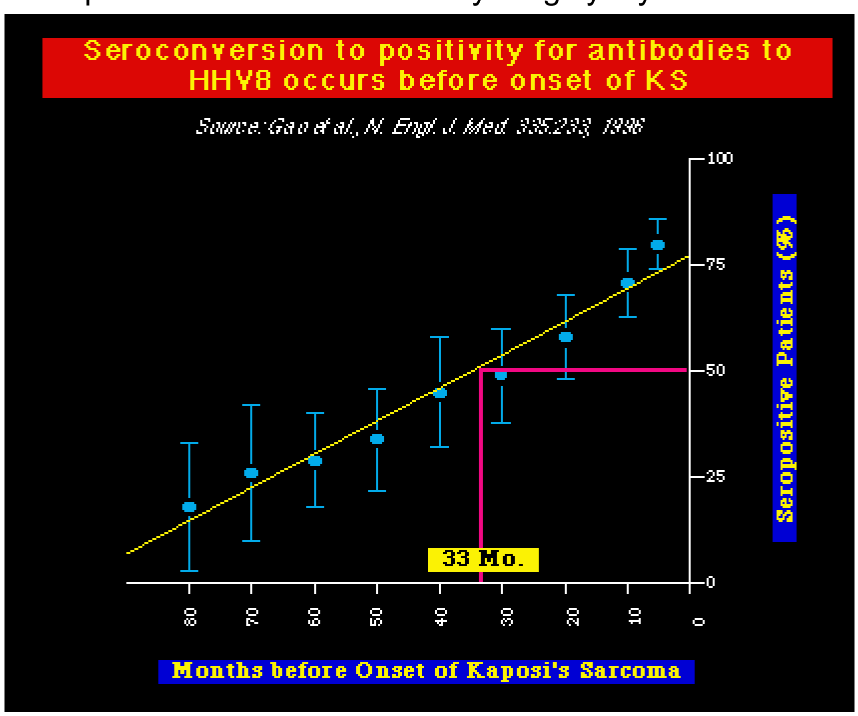


Some scientific evidence linking HHV-8 to KS.

Convincing evidence of the association of HHV-8 with KS has been provided by several studies. One of the first was conducted by Robin Weiss and colleagues, who used PCR to detect HHV-8 DNA in the blood of HIV-infected persons. They separated their subjects into two groups (HHV8+ at time zero, and HHV8- at time zero), and then asked the question: what is the rate of development of KS in the two groups? The result is shown below.



Additional evidence for a causal association between HHV-8 and KS has been provided by Moore and Chang, who used a different parameter to assess HHV-8 infection. In this case, they examined a group of HIV-infected individuals for the presence of antibodies against HHV-8. Then, they measured how long it took for KS to develop. On average, they found that seroconversion to positivity for HHV-8 predicted and preceded the onset of KS by roughly 3 years.

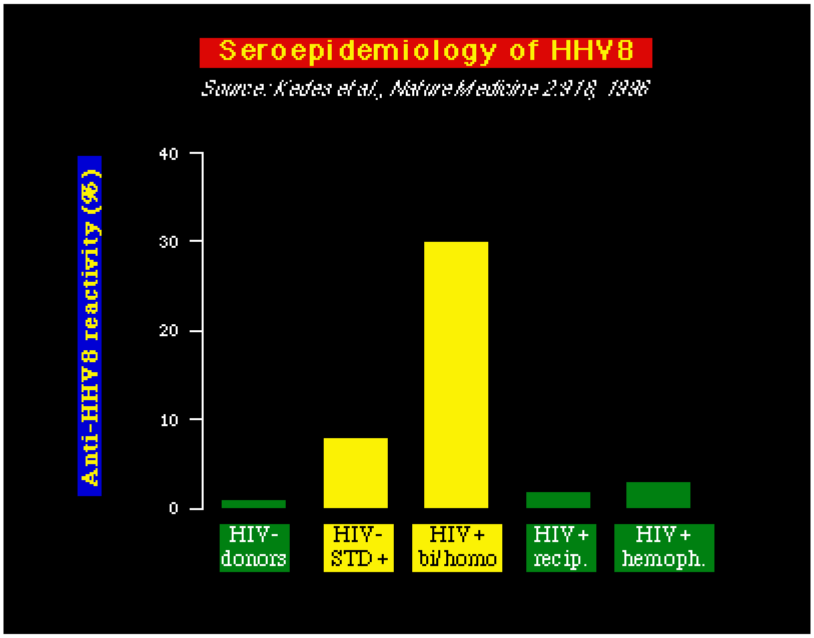


HHV-8 has also been found to be associated with certain types of B cell tumors in persons with AIDS, known as **primary effusion lymphomas** (aka *body-cavity B cell lymphomas*). Furthermore, the virus has been shown to preferentially **infect B cells**.

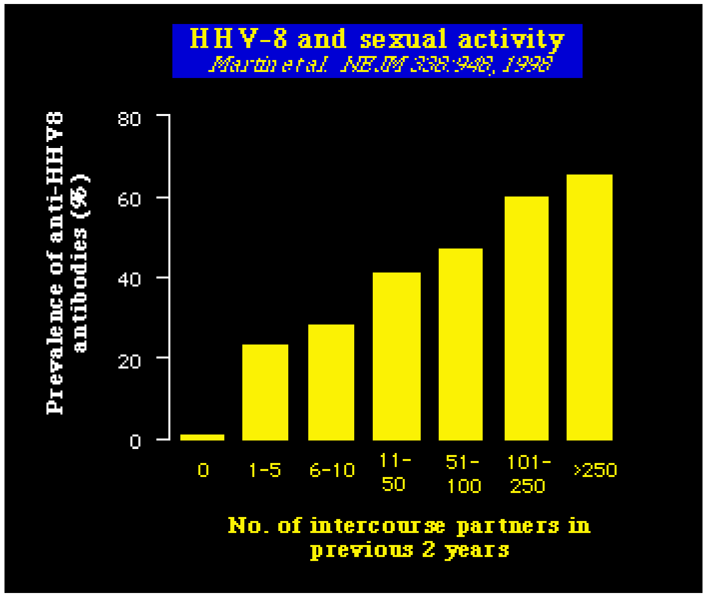
Virus Transmission

HHV-8 has been found in prostate tissue and in human semen, suggesting one possible means by which the virus may be transmitted. Additional data also support the idea that HHV-8 may be **sexually transmitted**:

First, Dean Kedes and colleagues surveyed blood samples for the presence of antibodies to HHV-8, and found that antibodies to HHV-8 were relatively common in HIV-negative persons who attended sexually-transmitted disease clinics (HIV-STD+), compared to HIV-negative blood donors (HIV- donors). In addition, and consistent with the epidemiology of the disease, HHV-8 was common in gay and bisexual men with HIV (who commonly develop KS), but much rarer in other groups of HIV-positive persons such as hemophiliacs (HIV+ hemoph.) or recipients of HIV-infected blood transfusions (HIV+ recip.), groups who rarely develop KS.



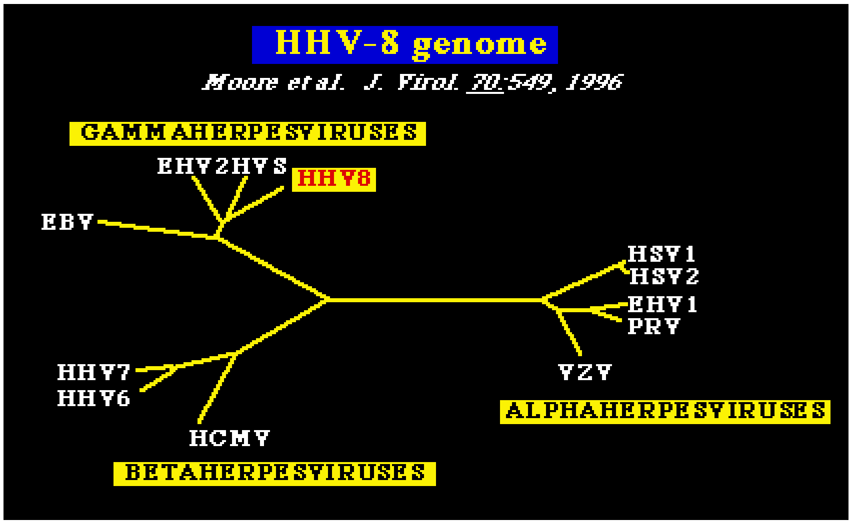
Second, this same group of investigators has also found that the risk of becoming infected with HHV-8 *(as defined by the presence of antiviral antibodies)* is directly related to the number of sexual partners a person has:



Other known risk factors for HHV-8 infection include oral-penile contact (oral exposure to infectious semen) and deep kissing with a virus-positive partner (oral exposure to infectious saliva); see below).

Important Note: Recent data (Pauk et al., N. Engl. J. Med. 343:1369, 2000) have shown that HHV-8 is also shed in saliva, at a very high rate. Deep kissing may lead to oral exposure to infectious saliva, and subsequent transmission of the virus. This may have important implications for Òsafe sexÓ practises. Also, since deep kissing and oral-penile contact are common among heterosexual couples, these findings also raise questions as to why male homosexual couples are at much increased risk for HHV-8 infection.

Viral Genetics, including Transforming Genes



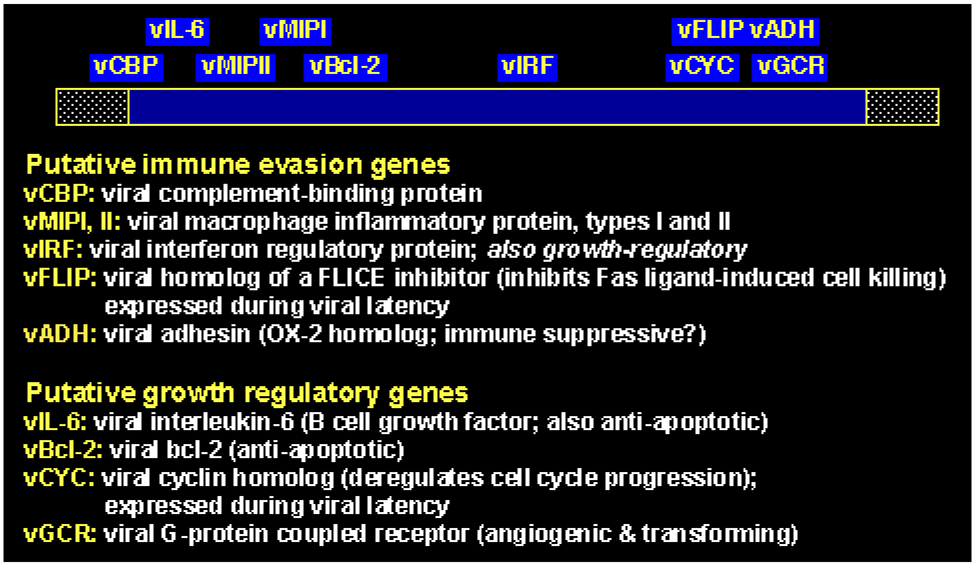
NB: HHV-8 is a Rhadinovirus, whereas EBV is a lymphocryptovirus.

In late 1996, the entire genome of HHV-8 was sequenced by several groups. The viral genome was found to contain several interesting genes, including homologs of various cellular genes (Moore et al. Science 274:1739, 1996), including:

- **Cytokines**: an interleukin-6 homolog (v-IL6), which is a functional B cell growth factor and a negative regulator of apoptosis
- **Chemokines:** Two viral chemokines with homology to MIP-1[alpha]. Both are angiogenic *in vitro*. In addition, v-MIP-II is a broadly active antagonist of cellular chemokines (see below) and can inhibit HIV-1 infection.
- **Cytokine response genes**: an interferon regulatory factor homolog. This can confer resistance to the anti-proliferative effects of interferon-alpha. It also interacts with the transcriptional coactivator protein p300 to alter gene expression, and it can upregulate c-myc expression, which may promote cell cycle progression.
- **Cellular receptors**: homologs of the complement receptor 2 (CR2/CD21), an adhesion molecule (NCAM) and a T-cell cell surface molecule with putative immunosuppressive properties (OX-2)
- **Growth regulatory genes**: a cyclin D homolog encoded by ORF72. This forms an active cyclin complex with cdk6, that is resistant to normal control pathways. As a result, the v-cyclin prevents normal G1 cycle arrest of cell proliferation.
- **G-protein coupled receptor:** ORF74 can stimulate signalling pathways linked to cellular proliferation, and can act as an oncogene *in vitro*; this gene product can also stimulate the release of the potent angiogenic factor, VEGF (vascular endothelial growth factor), in response to binding by cellular chemokines
- **Inhibitors of apoptosis**: a *bcl-2* homolog (v-*bcl-2*), which can inhibit apoptosis (programmed cell death); a FLICE-inhibitory protein (v-FLIP) encoded by ORF71, which can prevent Fas-mediated cell killing; *v-IL-6 (see above).*

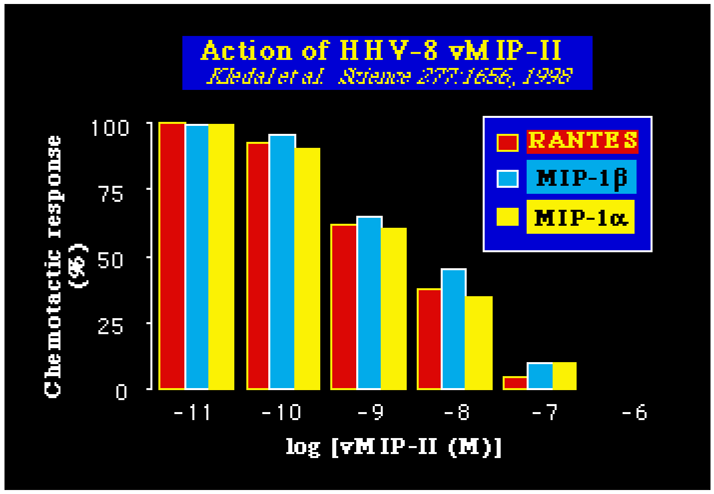
- **DNA synthesis and replication:** Like other herpesviruses, HHV-8 contains genes for ribonucleotide reductase (RR), dihydrofolate reductase (DHFR), thymidine kinase (TK) and thymidylate synthase (TS).

Selected viral genes involved in immune evasion or growth deregulation.



Examples of the activities of specific genes

Immune evasion genes: The viral chemokine homolog, v-MIP-II, has the unusual property that it can inhibit the normal action of a broad spectrum of cellular chemokines. These chemokines (including RANTES, MIP-1[alpha] and MIP-1[beta]; see below) normally help to recruit inflammatory cells to sites of infection, via chemotaxis. The unusually broad spectrum of activity of v-MIP-II makes it particularly interesting as a lead compound, from which to develop new anti-HIV drugs which can block the interaction between HIV and multiple chemokine receptors.



Growth regulatory genes: Like its normal cellular counterpart, the viral cyclin D homolog (**vCYC**) forms a complex with the cyclin-dependent kinase, CDK6. However, unlike cyclin D, the viral cyclin can efficiently prevent the normal G1 cycle arrest of cell proliferation.

The results of one experiment which demonstrated this effect are shown below. Briefly, 3T3 cells were engineered to contain an inducible expression cassette encoding either the viral cyclin (3T3/K cells), or nothing (3T3 cells). The cells were then growth arrested by depriving them of serum (serum contains growth factors necessary for the proliferation of cultured cells), and either treated with an inducer of vCYC synthesis (3T3/K+) or not treated (3T3/K-). After induction of vCYC production, the cell cycle status of the cells was analyzed by flow cytometry. The data show that wild-type 3T3 cells or 3T3/K cells which were not induced to express the viral cyclin remained arrested in the G1 phase of the cell cycle. In contrast, induction of vCYC expression resulted in escape from G1 arrest and progression of the cells through the cell cycle.

Effect of vCYC on cycling of growth arrested cells

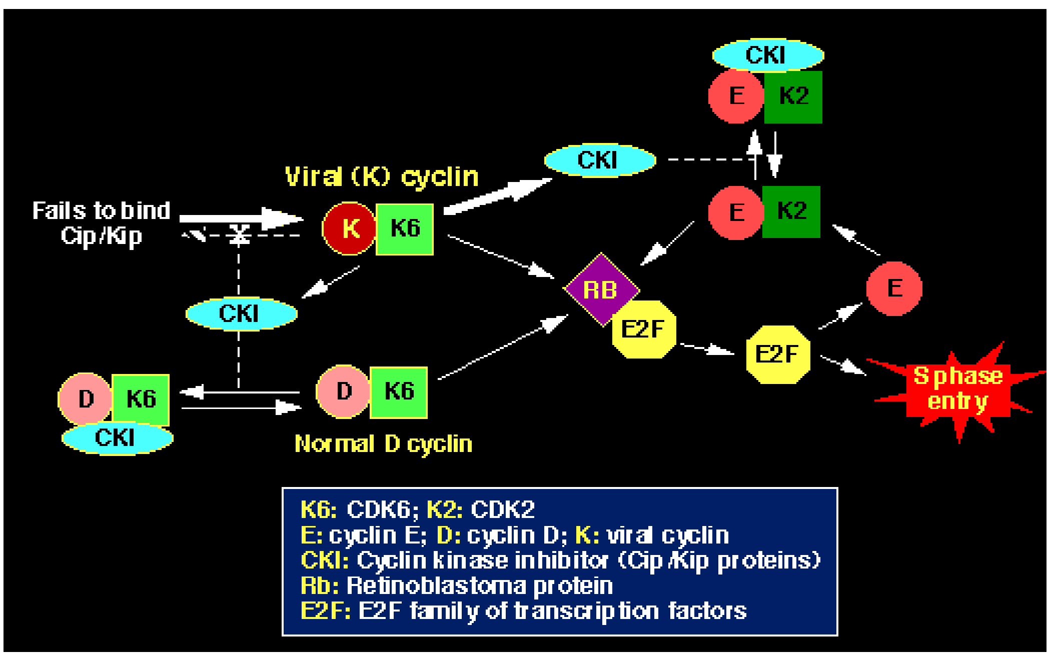
	3T3	3T3/K-	3T3/K+
%G1	93	89	69
%S	0.2	2.2	24
%G2/M	5.9	5.3	7.2

Swanton et al. Nature 390:184, 1997

There has been considerable effort to understand how it is that the viral cyclin is able to mediate its effects on cell cycle progression. The current view is the viral cyclin is able to overcome the normal regulatory circuits which suppress the activity of the cellular cyclins, cyclin E and cyclin D. This is thought to occur via two major mechanisms:

1. The complex between CDK6 (cellular cyclin-dependent kinase 6) and vCYC **fails to bind to CKIs** (these cyclin kinase inhibitors are the normal regulators of cyclin activity, and they include the Cip/Kip family of proteins). This is different from what happens with the normal cyclin D:CDK6 complex.
2. The vCYC:CDK6 complex has an extended substrate range, compared to the normal cyclin D:CDK6 complex. This **extended substrate range includes CKIs**, which are not normally substrates for the cyclin D:CDK6 complex. As a result, the vCYC:CDK6 complex phosphorylates and inactivates the CKI proteins. This results in the removal of the regulatory circuit that normally opposes the action of cyclin E (see below).

<u>Overview of the action of the action of the viral cyclin</u>



Abbreviations:

K6: CDK6; K2: CDK2 (CDK = cyclin-dependent kinase)

E: cyclin E; D: cyclin D; K: viral cyclin

CKI: Cyclin kinase inhibitor (Cip/Kip proteins)

Rb: Retinoblastoma protein; **E2F:** E2F family of transcription factors

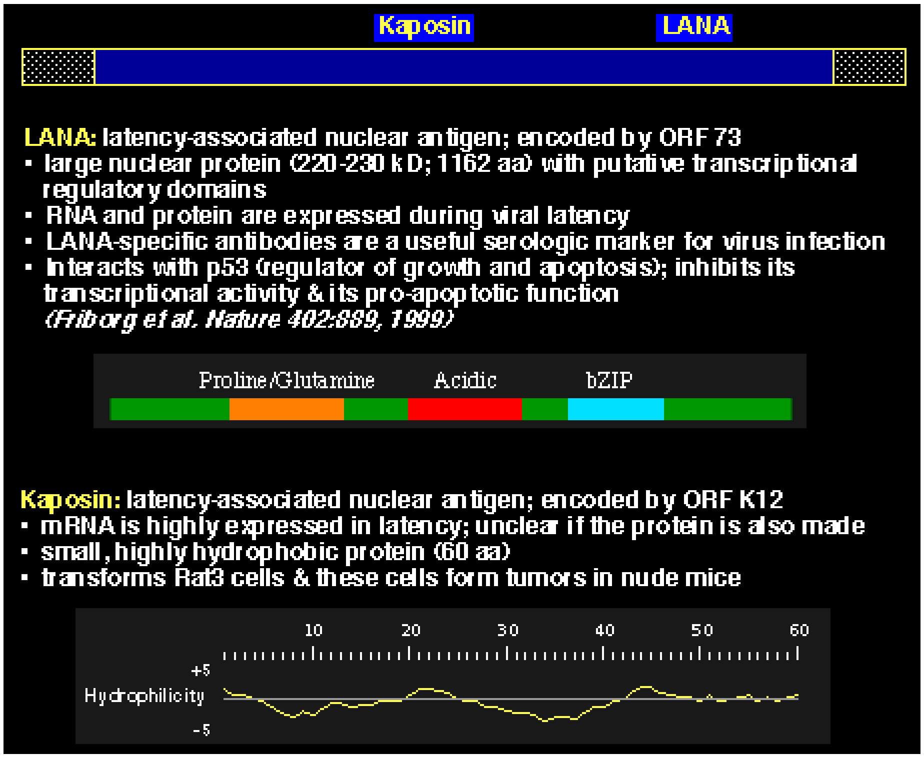
Explanation:

A major physiologic target for the cyclin D:CDK6 complex (and for the vCYC:CDK6 complex also) is the retinoblastoma protein, Rb. This is a potent tumor suppressor protein, which acts to prevent unscheduled cell cycle progression, by binding the E2F family of transcription factors. Phosphorylation of Rb cancels its ability to repress E2F, and as a result, the expression of genes required for entry into S phase is activated (E2F allows for the coordinated transcription of a group of genes whose actions are required for DNA synthesis). Among the genes switched on by E2F is cyclin E, which acts in concert with CDK2 to further phosphorylate Rb. The viral cyclin enhances this effect by removing the CKIs which would normally regulate the activity of this cyclin E:CDK2 complex.

Virus-specific genes involved in cellular transformation.

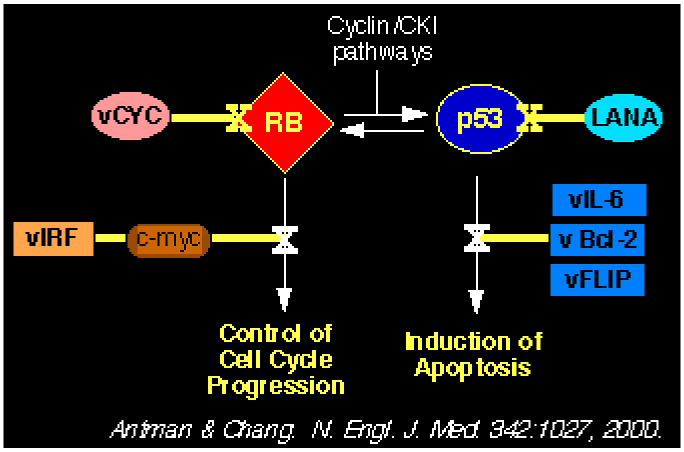
In addition to the various viral homologs of cellular genes, HHV-8 also encodes its own unique regulators of cell growth. Two of these are LANA and kaposin, both of which are thought to be expressed during the latent cycle of virus infection.

Overview of selected virus-specific genes with transforming potential.



Overall, the combined action of the various viral transforming genes allows the virus to subvert two of the most important regulators of cell growth -- the p53 protein and the retinoblastoma protein. Interestingly, the selective inhibition of these two key regulators is achieved not only by HHV-8 but also by small DNA viruses such as the transforming types of human papillomavirus.

Inhibition of p53 and Rb by HHV-8



Pathogenesis of KS and other tumors related to HHV-8

It is not clear which, if any, of the HHV-8 gene products may be important to the development of KS and other HHV-8 associated tumors (see below). Indeed, the pathogenesis of KS remains poorly understood and is likely to be complex (Gallo, Science 282:1837, 1998). Key features of KS include:

1. The lack of an histologically obvious neoplastic cell, suggesting that the disease is not neoplastic *per se*, but rather a hyperproliferative and highly vascularized cell mass.
2. The presence of latent HHV-8 infection in the majority of the spindle cells which make up the KS lesion. These spindle cells secrete angiogenic and growth factors, and are thought to provide the driving force for the development of KS. Only a very low percentage of these cells (1-5%) actually make lytic-cycle HHV-8 gene products (such as the growth regulatory proteins, chemokines, etc). *Note: latent genes of HHV-8 with transforming activity include the ORF71-73 gene products (vFLIP, vCYC and LANA) as well as (possibly) the ORK K12 product (kaposin).*
3. A huge increase (by 20,000 to 50,000 fold) in the incidence of KS in association with HIV infection. This suggests that cofactors, or HIV proteins, may play a role in KS