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Review

# Fundamental and accessory systems in herpesviruses

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**Abstract**

Evolutionary studies have a large theoretical component and will not directly provide therapies for herpesvirus infections. However, they do provide a conceptual framework within which we can evaluate the origins of the various systems that contribute to viral lifestyle. An evolutionary context allows ancient systems that are fundamental to the replication of all herpesviruses to be distinguished from those that have developed relatively recently in order to tailor viruses to particular biological niches. Both categories are in principle accessible to intervention, either to prevent basic replicative capabilities or to reduce the advantages that the virus has in its interactions with the host. Phylogenetic data provide estimates of evolutionary rate for herpesviruses that are only between one and two orders of magnitude greater than those of their hosts. However, it is becoming apparent that certain genes have evolved much faster under selection pressures and by mechanisms that are not well understood. Nonetheless, the mutation rates of even the most highly conserved genes are sufficient to permit herpesviruses to escape from antiviral therapy. Greater understanding of the origins and functions of herpesvirus genes may lead to new insights into the determinants of pathogenesis and hence to new diagnostic and therapeutic targets. 

*Keywords:* Herpesviruses; Evolution; HCMV; DNA polymerase

## 1. Evolutionary pathways

Complete genome sequences are presently available for 28 herpesvirus species, including all of the eight known human herpesviruses (Table 1). With the exception of channel catfish virus, these belong to the three subfamilies that encompass the mammalian and avian herpesviruses, and are spread across the genera that make up these subfamilies. This weight of data has enabled a more extensive examination of the relationships between, and thus the evolution of the herpesviruses than has been achieved for any other family of large DNA viruses. The level of activity is such that in future not only will more herpesvirus species be sequenced, but also multiple strains of the more prominent species, as is now the situation for smaller viruses. Indeed, two or three strains have already been sequenced for several species (for example, human herpesviruses 6 and 8; HHV-6 and HHV-8). The availability of sequences for multiple strains in coming years will make it possible to evaluate in detail the evolution

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Table 1
Herpesviruses for which complete genome sequences are available

| | |
|---|---|
| ***Alphaherpesvirinae*** | |
| *Simplexvirus* ($\alpha_1$) | |
| Herpes simplex virus type 1 | 152 261 |
| Herpes simplex virus type 2 | 154 746 |
| *Varicellovirus* ($\alpha_2$) | |
| Varicella-zoster virus | 124 884 |
| Simian varicella virus | 124 139 |
| Equine herpesvirus 1 | 150 224 |
| Equine herpesvirus 4 | 145 597 |
| Bovine herpesvirus 1 | 135 301 |
| 'Marek's disease-like viruses' ($\alpha_3$) | |
| Marek's disease virus type 1 | 177 874 |
| Marek's disease virus type 2 | 164 270 |
| Turkey herpesvirus | 159 160 |
| ***Betaherpesvirinae*** | |
| *Cytomegalovirus* ($\beta_1$) | |
| Human cytomegalovirus | 230 285 |
| Chimpanzee cytomegalovirus | 241 087 |
| *Muromegalovirus* | |
| Murine cytomegalovirus | 230 278 |
| Rat cytomegalovirus | 229 896 |
| *Roseolovirus* ($\beta_2$) | |
| Human herpesvirus 6 (3 strains) | 159 321 |
| | 161 573 |
| | 162 114 |
| Human herpesvirus 7 (2 strains) | 144 861 |
| | 153 080 |
| Unassigned | |
| Tupaiid herpesvirus 1 | 195 857 |
| ***Gammaherpesvirinae*** | |
| *Lymphocryptovirus* ($\gamma_1$) | |
| Epstein–Barr virus | 184 113 |
| Rhesus lymphocryptovirus | 171 096 |
| *Rhadinovirus* ($\gamma_2$) | |
| Herpesvirus saimiri | 112 930/1444 |
| Herpesvirus ateles | 108 409/1582 |
| Alcelaphine herpesvirus 1 | 130 608/1113 |
| Human herpesvirus 8 (2 strains) | ~ 140 500/801 |
| | ~ 140 500/801 |
| Rhesus rhadinovirus (2 strains) | 131 364/ ~ 2100 |
| | 130 733/[a] |
| Murine herpesvirus 68 (1 strain twice) | 118 237/1213 |
| | 118 311/1239 |
| Bovine herpesvirus 4 | 108 873/2267 |
| Equine herpesvirus 2 | 184 427 |
| ***Undefined subfamily*** | |
| 'Ictalurid herpes-like virus' | |
| Channel catfish virus | 134 226 |

Classification into subfamily and genus is indicated, with genome size (bp). *Rhadinovirus* genomes other than that of EHV-2 comprise a unique region flanked by a variable number of terminal repeats. The sizes of these two components are indicated.

[a] Unknown.

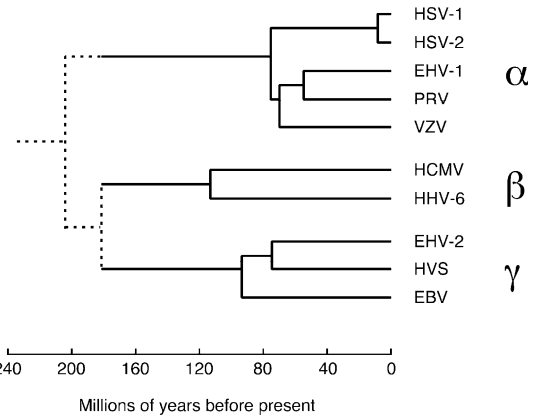


Fig. 1. Phylogenetic tree for mammalian herpesviruses in the *Alpha*-, *Beta*- and *Gammaherpesvirinae* genera (abbreviated as α, β and γ) based on conservation of amino acid sequences from several core genes. A proposed timescale is shown, with the oldest part of the tree shown as a broken line to indicate lower confidence. Additional abbreviations: EHV-1 and -2, equine herpesviruses 1 and 2; PRV, pseudorabies virus; VZV, varicella-zoster virus; HVS, herpesvirus saimiri. Adapted from McGeoch et al. (1995) with permission of the publisher Academic Press.

of species over the relatively short term (that is, within the scale of human history), and complement the current view of evolution over the much longer term.

Mammalian and avian herpesviruses have between about 70 and 220 genes (McGeoch and Davison, 1999). Phylogenetic comparisons between related genes are usually conducted at the level of primary amino acid sequence because of the generally high degree of nucleotide sequence divergence, and often focus on genes that are highly conserved in all herpesviruses (for example, those encoding DNA polymerase and glycoprotein B). In a seminal study, McGeoch and Cook (1994) showed that the phylogeny of members of the *Alphaherpesvirinae* mirrors that of their hosts. This supports the view that herpesviruses have evolved with their hosts and have not frequently become established in new host species by transmission from the natural host. This correlation allowed an estimation of evolutionary rate for the herpesviruses, which turned out to be very approximately 30 times faster than that of the hosts. Although herpesviruses have evolved at a rate several orders of magnitude less than that for some RNA viruses, such as HIV-1 and influenza A virus

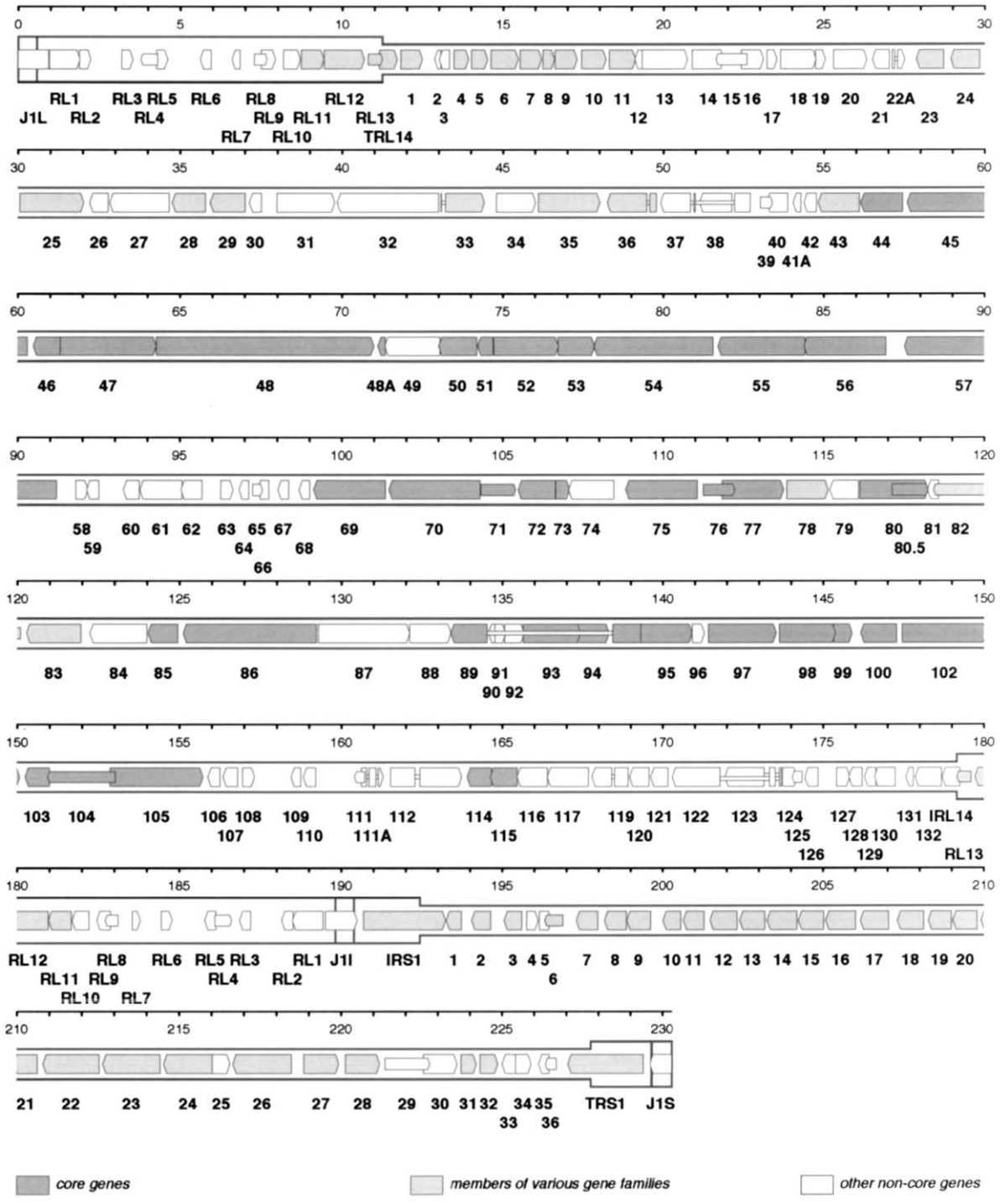
0 5 10 15 20 25 30
RL1 RL3 RL5 RL6 RL8 RL12 1 2 4 5 6 7 8 9 10 11 13 14 15 16 18 19 20 22A 24
J1L RL2 RL4 RL9 RL11 RL13 3 12 17 21 23
RL7 RL10 TRL14
30 35 40 45 50 55 60
25 26 27 28 29 30 31 32 33 34 35 36 37 38 40 42 43 44 45
39 41A
60 65 70 75 80 85 90
46 47 48 48A 49 50 51 52 53 54 55 56 57
90 95 100 105 110 115 120
58 60 61 62 63 65 67 69 70 71 72 73 74 75 76 77 78 79 80 81 82
59 64 68 80.5
66
120 125 130 135 140 145 150
83 84 85 86 87 88 89 91 93 94 95 96 97 98 99 100 102
90 92
150 155 160 165 170 175 180
103 104 105 106 108 109 111 112 114 116 117 119 121 122 123 124 127 131 IRL14
107 110 111A 115 120 125 128 130 132
126 129 RL13
180 185 190 195 200 205 210
RL12 RL8 RL6 RL5 RL3 RL1 J1I IRS1 1 2 3 4 5 7 8 9 10 11 12 13 14 15 16 17 18 19 20
RL11 RL9 RL4 RL2 6
RL10 RL7
210 215 220 225 230
21 22 23 24 25 26 27 28 29 30 31 32 34 35 TRS1 J1S
33 36
core genes
members of various gene families
other non-core genes

Fig. 2

(Sharp, 2002), their mutation rate is sufficient to allow herpesviruses to escape from therapeutic regimes by selection of resistant mutants, though such mutants have not entered into evolutionary lineages (Coen, 1991; Crumpacker, 2001). McGeoch et al. (1995) showed that application of this rate to the family as a whole indicates that certain of the major divergences resulting in present-day herpesviruses occurred at the time of the mammalian radiation, some 60–80 million years ago (Fig. 1). Further extrapolation implied tentatively that mammalian and avian herpesviruses evolved from a common ancestor that existed about 200 million years ago, a period when early mammals were in existence. This ancestor contributed 43 genes (termed core genes) to present-day herpesviruses (McGeoch and Davison, 1999). A reliable phylogeny is now available for 46 herpesvirus species (McGeoch et al., 2000).

As would be expected, herpesviruses have exploited all the mechanisms available for generating diversity (McGeoch and Davison, 1999; Davison, 2001). These include the cumulative effects of the substitution, deletion or insertion of nucleotides, generation of genes de novo (for which there is very limited evidence), duplication of genes by recombination followed by functional divergence, capture of genes from the host genome usually via an RNA intermediate, and large scale gene rearrangement. The effects of some of these mechanisms are apparent in the largest herpesvirus, human cytomegalovirus (HCMV; formally human herpesvirus 5), a member of the *Betaherpesvirinae*. Fig. 2 shows the gene arrangement in the AD169 laboratory strain of HCMV proposed by Chee et al. (1990) and modified by data published subsequently. HCMV possesses 40 of the core genes. Many non-core genes are related to each other in gene families that have arisen via gene duplication. Also, several genes have been captured from the host during evolution. These include ancient relics such as the UL54 DNA polymerase gene and recent additions such as the UL111A viral interleukin-10 gene. The AD169 laboratory strain of HCMV has been passaged many times in fibroblast cell culture. It is now known to lack a 15 kilobase pair (kbp) region at the 179 kbp location, and exhibits a number of other differences compared to low passage strains of the virus (Cha et al., 1996). Consequently, wild type HCMV contains at least 19 extra genes that are not present in AD169.

## 2. Fundamental systems

As indicated above, herpesvirus genes may be divided into two categories: core genes that were inherited from a common ancestor and thus form ancient systems that are fundamental to replication, and non-core genes that developed more recently and thus represent accessory systems that fit a virus to a particular biological niche. The following discussion will consider these two categories in greater detail.

Table 2 lists the 43 core genes, sorted into their functional categories. Three genes that are ancestral to UL9, UL23 and UL40 in herpes simplex virus type 1 (HSV-1, formally human herpesvirus 1) have subsequently been lost from certain lineages. Core genes are involved mainly in the structure and assembly of the viral capsid, egress of capsids from the nucleus, DNA replication and packaging and, to a lesser extent, the biochemistry peripheral to DNA replication. The fact that these systems have been conserved implies that they were employed by an ancestral herpesvirus some 200 million years ago.

The products of some core genes are minor components of the tegument (the proteinaceous

Fig. 2. Layout of predicted protein-coding ORFs in the genome of HCMV strain AD169 (Chee et al., 1990 and modified subsequently). Changes to this picture emerging from comparative genomics have not been incorporated, including removal of a significant number of small non-core genes as being unlikely to encode proteins and discovery of several new genes (Davison, A.J., Dolan, A., Akter, P., Addison, C., Dargan, D.J., Alcendor, D.J., McGeoch, D.J., Hayward, G.S., unpublished data). The scale is in kbp, and inverted repeat regions are shown in a thicker format than unique regions. Protein-coding regions are indicated by arrows grouped according to the key, with gene nomenclature below. Introns are shown as narrow white bars. Genes in the inverted repeats are given their full nomenclature, but the UL and US prefixes have been omitted from genes in the unique regions (UL1-UL132 at 12–178 kbp and US1-US36 at 193–227 kbp).

Table 2
Properties of core herpesvirus genes

| Functional category | HSV-1 | HCMV | HHV-8 | Function |
|---|---|---|---|---|
| Capsid assembly and structure | UL19 | UL86 | 25 | Major capsid protein; component of hexons and pentons |
| | UL18 | UL85 | 26 | Component of intercapsomeric triplex |
| | UL38 | UL46 | 62 | Component of intercapsomeric triplex |
| | UL35 | UL48A | 66 | Located on tips of hexons |
| | UL26 | UL80 | 17 | Protease and minor capsid scaffold protein |
| | UL26.5 | UL80.5 | 17.5 | Major capsid scaffold protein |
| DNA replication machinery | UL9 | – | – | Binds to origins of DNA synthesis; helicase; *Alphaherpesvirinae* and genus *Roseolovirus* of the *Betaherpesvirinae* only |
| | UL30 | UL54 | 9 | Catalytic subunit of DNA polymerase; complexes with UL42 protein in HSV-1 |
| | UL42 | UL44 | 59 | Processivity subunit of DNA polymerase; complexes with UL30 protein in HSV-1 |
| | UL5 | UL105 | 44 | Component of DNA helicase–primase complex; helicase |
| | UL8 | UL102 | 41 | Component of DNA helicase–primase complex |
| | UL52 | UL70 | 56 | Component of DNA helicase–primase complex; primase |
| | UL29 | UL57 | 6 | Single-stranded DNA-binding protein |
| Peripheral enzymes | UL23 | – | 21 | Thymidine kinase; *Alpha*- and *Gammaherpesvirinae* only |
| | UL39 | UL45[a] | 61 | Ribonucleotide reductase; large subunit |
| | UL40 | – | 60 | Ribonucleotide reductase; small subunit; *Alpha*- and *Gammaherpesvirinae* only |
| | UL50 | UL72[a] | 54 | Deoxyuridine triphosphatase |
| | UL2 | UL114 | 46 | Uracil-DNA glycosylase |
| Processing and packaging of DNA | UL12 | UL98 | 37 | Deoxyribonuclease; role in DNA maturation |
| | UL15 | UL89 | 29 | Putative ATPase subunit of terminase |
| | UL28 | UL56 | 7 | Putative subunit of terminase |
| | UL6 | UL104 | 43 | Putative portal protein |
| | UL25 | UL77 | 19 | |
| | UL32 | UL52 | 68 | |
| | UL33 | UL51 | 67A | |
| | UL17 | UL93 | 32 | Tegument protein |
| Egress of capsids from nucleus | UL31 | UL53 | 69 | Nuclear matrix protein; interacts with UL34 protein in HSV-1 |
| | UL34 | UL50 | 67 | Inner nuclear membrane protein; interacts with UL31 protein in HSV-1 |
| Tegument | UL7 | UL103 | 42 | |
| | UL11 | UL99 | 38 | Myristylated protein |
| | UL14 | UL95 | 34 | |
| | UL16 | UL94 | 33 | |
| | UL36 | UL48 | 64 | |
| | UL37 | UL47 | 63 | |
| | UL51 | UL71 | 55 | |
| Surface and membrane | UL27 | UL55 | 8 | Glycoprotein B |
| | UL1 | UL115 | 47 | Glycoprotein L; complexes with glycoprotein H |
| | UL22 | UL75 | 22 | Glycoprotein H; complexes with glycoprotein L |
| | UL10 | UL100 | 39 | Glycoprotein M; complexes with glycoprotein N |
| | UL49A | UL73 | 53 | Glycoprotein N; complexes with glycoprotein M |
| Control and modulation | UL13 | UL97 | 36 | Serine-threonine protein kinase; tegument protein |
| | UL54 | UL69 | 57 | Post-transcriptional regulator of gene expression |
| Unknown | UL24 | UL76 | 20 | |

[a] Probably lacks activity shown.

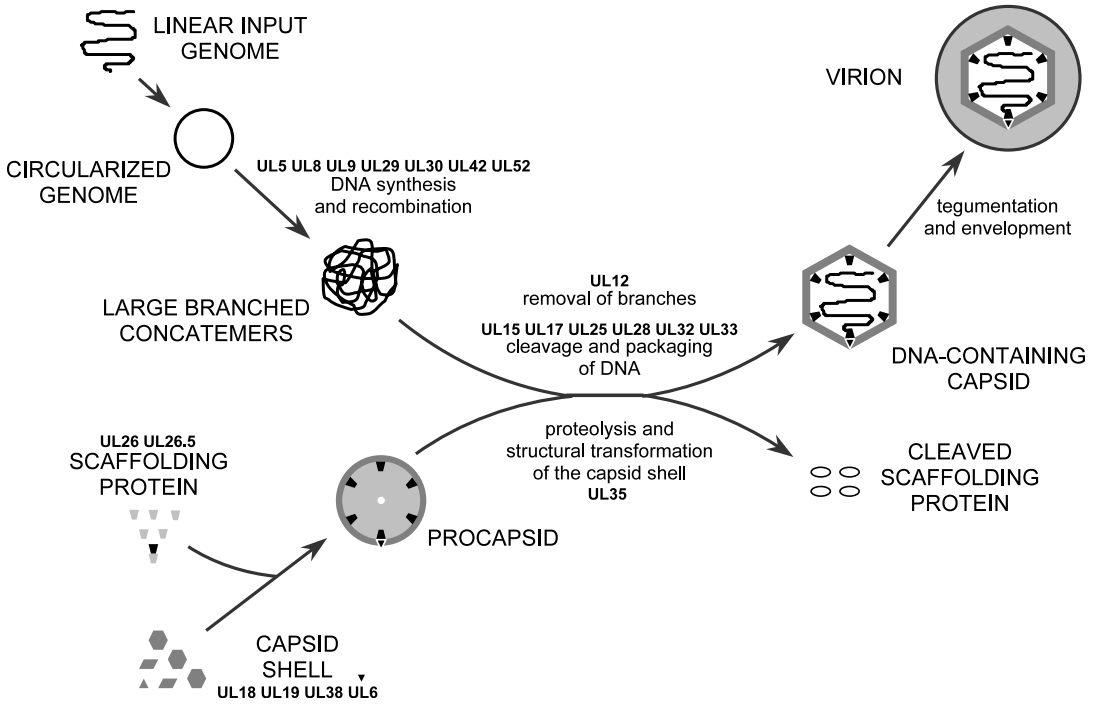


Fig. 3. Cartoon showing processes involved in HSV-1 genome replication and virion assembly.

layer surrounding the capsid) or in the viral envelope. Two proteins that function in control processes are also specified by core genes: one involved in post-transcriptional regulation and the other in protein phosphorylation. Inclusion of the latter in the control category is nominal, however, as its physiological role is not well understood. The protein kinase is serendipitously responsible for phosphorylation of ganciclovir by HCMV (Littler et al., 1992). Many genes in the tegument, surface and control categories do not appear in Table 2 because they are more recent evolutionary additions.

The proteins that make up the capsid are encoded by core genes (Homa and Brown, 1997). The majority of the capsid shell in the icosahedral HSV-1 capsid is made up of the major capsid protein (specified by UL19) arranged in 150 hexons and 12 pentons. In between these structures lie the triplexes, each of which consists of two copies of one protein (UL18) and one copy of another (UL38). The structure of the herpesvirus capsid is characteristic not only of herpesviruses of mammals, but also of those of fish and even of one herpesvirus of an invertebrate, the oyster (Booy et al., 1996; Davison, A.J., Trus, B.L., Steven, A.C., Watson, M.S., Le Deuff, R.M., Renault, T., unpublished data). This suggests that the herpesvirus capsid structure is very ancient, probably having been in existence for at least a billion years. The ultimate origins of the capsid protein genes are unknown. If they came from cellular genes, adaptation to their current usage has obliterated any clue to original function.

Fig. 3 shows how the fundamental systems of herpesvirus capsid formation and DNA replication merge, using HSV-1 as an example. The incoming viral genome makes its way to the nucleus, becoming circularised probably through the action of cellular ligases, commences gene expression and initiates DNA synthesis by the activities of seven viral proteins (Wu et al., 1988). These proteins are the two-subunit DNA polymerase holoenzyme (UL30 and UL42), the three-subunit DNA helicase–primase (UL5, UL8 and UL52), the single-stranded DNA-binding protein

(UL29) and a protein that recognises the origins of DNA synthesis (UL9) (Lehman and Boehmer, 1999). The means by which the origins are recognised appears to have been inherited by the *Alphaherpesvirinae* and the *Roseolovirus* genus of the *Betaherpesvirinae*, but other herpesviruses lack a counterpart of UL9. The capsid is assembled in the nucleus around a scaffold that consists of the major scaffolding protein (UL26.5) plus a version of this protein, which has a protease domain at the amino terminus (UL26). This gives rise to a transient, roughly spherical procapsid consisting of the capsid shell proteins arranged around the scaffold (Homa and Brown, 1997).

The products of DNA replication and concurrently occurring intra- and inter-molecular recombination are large branched head-to-tail concatemers, in which branches are resolved through the action of a DNase (UL12) (Goldstein and Weller, 1998). After this, several events take place in a tightly coupled process. The concatemers are cleaved into unit length genomes and packaged to high density into procapsids in which enzymatic steps catalysed by the protease are associated with loss of the scaffold and angularization of the capsid. The protease domain cleaves itself from the scaffolding domain and also at a site near the C terminus of the scaffolding domain of the UL26 and UL26.5 proteins, to allow release of the scaffolding protein from the capsid. At the end of the process, the protease domain remains associated with the capsid. A small protein (UL35) also becomes associated with the tips of the hexons.

The functions of the seven viral proteins involved in cleavage and packaging of DNA have been only partially characterised. The UL15 protein is a putative ATPase, and is the most highly conserved gene in herpesviruses (Davison, 1992). It associates with the UL28 protein to form the presumptive terminase responsible for inserting DNA into the capsid and cleavage of concatemers into unit-length genomes (Koslowski et al., 1999). An association of the UL33 protein with this complex has also been reported (Beard et al., 2002). The UL6 protein forms the putative portal through which the DNA is inserted, and remains associated with capsids during subsequent steps (Newcomb et al., 2001).

Egress of the capsid from the nucleus involves the UL31 and UL34 proteins (Mettenleiter, 2002). Subsets of tegument proteins are added in the nucleus or cytoplasm, a membrane and its associated glycoproteins are acquired by budding into a post-Golgi compartment, and mature virions leave the cell by exocytosis (Mettenleiter, 2002). Certain aspects of these later stages of tegumentation and envelopment involve core genes, but not all.

In summary, the fundamental systems for herpesvirus replication involve several crucial enzymatic steps and many interactions between viral proteins or between viral proteins and DNA. These processes represent obvious targets for inhibition. Indeed, many current antiherpetic agents operate against parts of these systems; for example the catalytic subunit of DNA polymerase via phosphorylation by the thymidine kinase (acyclovir: Fyfe et al., 1978) or protein kinase (ganciclovir: Littler et al., 1992), or the terminase (benzimidazole ribonucleosides: Underwood et al., 1998; Krosky et al., 1998) or the helicase–primase complex (amino-thiazolylphenyl-containing compounds and thiazole urea derivatives: Crute et al., 2002; Kleymann et al., 2002). It is likely that additional points of intervention in these central processes will be found as the mechanisms become understood in greater detail.

## 3. Accessory systems

Accessory systems allow particular viruses to occupy biological niches and to overcome barriers put up by the host. They fall into four main categories. The first concerns functions that give the virus specific cellular tropisms. Different herpesviruses have different host ranges, and herpesviruses that infect the same host can infect different types of cell within that host. Although we have some information about the viral genes involved in tropism, for example in EBV (Faulkner et al., 2000) and HCMV (Sinzger et al., 1999), this is a complex area where most of the important discoveries are yet to be made. The second

Table 3
HCMV accessory genes involved in manipulating the apoptotic or immune responses

| | |
|---|---|
| UL16 | Inhibits sensitization to NK cells |
| UL18 | MHC-I homologue |
| UL33 | Putative chemokine receptor |
| UL36 | Cell death suppressor, binds to caspase-8 and inhibits Fas-mediated apoptosis |
| UL37 | Exon 1 encodes a mitochondrial inhibitor of apoptosis |
| UL40 | Contains HLA-E-binding peptide, upregulates HLA-E to evade NK cells |
| UL78 | Putative chemokine receptor |
| UL111A | IL-10-like cytokine, potent immunosuppressive |
| UL144 | TNFR homologue |
| UL146 | IL-8-like α-chemokine, chemotactic for neutrophils |
| UL147 | Putative chemokine |
| US2 | Targets MHC-I for proteasome-dependent degradation; down-regulates MHC-II |
| US3 | Promotes retention of MHC-I in the ER |
| US6 | Inhibits TAP-mediated peptide translocation |
| US11 | Targets MHC-I for proteasome-dependent degradation |
| US27 | Putative chemokine receptor |
| US28 | Chemokine receptor, binds human β-chemokines |

category concerns functions that give the virus a degree of control over cellular processes. These include proteins that affect transcription and translation of viral genes at the expense of the host, some of which have been examined in detail. Recently, it has become clear that herpesviruses have evolved ways of controlling the apoptotic defences of the host, the best studied cases involving several viral genes (Hay and Kannourakis, 2002).

The third category, in which the sophistication of herpesviruses is being increasingly appreciated, is their ability to manipulate or evade the host immune system, particularly in the case of HCMV (Hengel et al., 1998; Lehner and Wilkinson, 2001). Studies in this area also shed light on the mechanisms and relative importance of the various immune defences, and it is certain that many more interesting discoveries in viral gene function will be made in this respect. The fourth accessory system is that of latency, which is a feature of all herpesviruses. It appears that the molecular systems for latency used by herpesviruses differ widely from virus to virus, indicating replacement during evolution (Davison and McGeoch, 1995). However, little is understood about how latency works at the molecular level, particular in the *Alpha*- and *Betaherpesvirinae*.

HCMV has the largest known genome among the herpesviruses, and the majority of its genes fall into the non-core (accessory) class (Fig. 2). These include the gene families, members of which are likely to share similar functions, each vital to the natural life cycle of the virus. Many of these genes are relatively recent in evolutionary terms, some having been added in the last few million years. Table 3 shows a list of HCMV accessory functions that are involved in regulation of apoptosis or manipulation of the immune system. Some (perhaps many) of these genes have been captured from the cellular repertoire and modified in various ways to be used against the host. An impressive range of captured functions also characterises HHV-8 (Neipel et al., 1997).

## 4. Genetic variation

Sequencing technology has enabled intense studies of genome variation in small viruses with a high impact on human health, such as HIV, hepatitis B and C viruses and influenza virus. Much is known about how these viruses vary and the timescale over which variation occurs. The power of sequencing is now so great that pan-genomic studies of variation in larger viruses such as the herpesviruses will be a logical development. This will make it possible to investigate whether particular strains of virus are associated with different types of disease. It will also allow identification of variable genes and yield insights potentially leading to diagnostic and therapeutic targets in the accessory category of genes.

Certain genes in members of the *Betaherpesvirinae* are particularly variable. Genes that exhibit low variation between HCMV strains include those involved in fundamental virus systems, such as the UL54 DNA polymerase (Chou et al., 1999) and the UL97 protein kinase (Chou et al., 1995). Some accessory genes are also well conserved, but others are more variable, such as part of the UL37 glycoprotein (Hayajneh et al.,

(a)

```
Toledo      MKPLIMLICFAVILLQLGVTKVCQHNEVQLGNECCPPCGSGQRVTKVCTD
PT1         MKPLVMLILLSVLLACIGKTEICKPEEVQLGNQCCPPCKQGYRVTGQCTQ
PT16        MKPLVMLICFGVFLLQLGGSKMCKPDEVKLGNQCCPPCGSGQKVTKVCTE
con         MKPL-MLI---V-L---G----C---EV-LGN-CCPPC--G--VT--CT-

Toledo      YTSVTCTPCPNGTYVSGLYNCTDCTQCNVTQVMIRNCTSTNNTVCAPKNH
PT1         YTSTTCTLCPNGTYVSGLYNCTNCTECNDTEVTIRNCTSTNNTVCASKNY
PT16        NSGITCTLCPNGTYLTGLYNCTNCTQCNDTQITVRNCTSTNNTICASKNH
con         ----TCT-CPNGTY--GLYNCT-CT-CN-T----RNCTSTNNT-CA-KN-

Toledo      TYFSTPGVQHHKQRQQNHTAHITVKQGKSGRHTLAWLSLFIFLVGIILLI
PT1         TSFSISGVQHHKQRQ.NHTAHVTVKQGKSGRHTLAWLSLFIFLVGIILLI
PT16        TSFSTLGVQHHKQRQQNHTAHVTVKQRKGGRHTLAWLSLFIFLVGIILLI
con         T-FS--GVQHHKQRQ-NHTAH-TVKQ-K-GRHTLAWLSLFIFLVGIILLI

Toledo      LYLIAAYRSERCQQCCSIGKIFYRTL
PT1         LYLIAAYRSEKCQQCCSIGKIFYRTL
PT16        LYLIAAYRSERCQQCCSIGKIFYRTL
con         LYLIAAYRSE-CQQCCSIGKIFYRTL
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(b)

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Toledo      MRLIFGALIIFL.AYVYHYE.VNGTELRCRCLHRKWPPNKIILGNYWLHR
Towne       MRLIFGSLISLLMAFMYYHG.VHSRELRCPCTHKAL..HHPIGGLFWVGR
C6          MRFIFGLLISLMVAHTCNAGLGSEGNGRCTCVGYHRFDKQLPRGTIWLGH
con         MR-IFG-LI----A-------------RC-C------------G--WL--

Toledo      DPRGP.GCDKNEHLLYPDGRKPPGPGVCLSPDHLFSKWLD...KHNDNRW
Towne       DPPNPPECDKPQHYLLP....PRGKPVCLAPDHHLSKWLD...GKKDNSW
C6          RPPGP.HCPRGDVLMKL....GEQPTVCLSDHHPLSKWMYRHHGSDTEIW
con         -P--P--C------------------VCL---H--SKW-----------W

Toledo      YNVNIT..KSPGPRRINITLIGVRG
Towne       HRVLVKVKDSNGPHVEENAVTNKRPRWK
C6          FQIEFK.....GPQNTKVVSKSFTPPS
con         -----------GP------------
```

Fig. 4. Amino acid sequence alignments of proteins from HCMV isolates. (A) UL144 TNFR homologues (transmembrane domain underlined). The sequences were obtained from: Toledo, Cha et al. (1996); PT16 and PT1, Lurain et al. (1999). (B) UL146 α-chemokine homologues. The sequences were obtained from: Toledo and Towne, Cha et al. (1996); C6, Prichard et al. (2001). Fully conserved residues are indicated in the 'con' line.

2001) and the UL144 homologue of the tumour necrosis factor receptor (TNFR; Lurain et al., 1999). The most variable gene yet discovered in the herpesviruses is HCMV UL146, which encodes an α-chemokine (Prichard et al., 2001).

Proteins with low variability differ by the very occasional amino acid residue. Fig. 4A shows an alignment between amino acid sequences for the moderately variable UL144 TNFR protein from three of the most divergent HCMV strains for which data are available. The variable region is in the ectodomain of this glycoprotein. Fig. 4B shows a similar alignment for the highly variable UL146 α-chemokine for three divergent strains. Most residues differ between the proteins. Although this level of variation would not be unusual in fast evolving viruses such as HIV, in a herpesvirus it is astounding. The nature of the evolutionary

pressures driving the change, and the mechanisms by which it occurs, are not fully understood, although immune selection may play a prominent part.

## 5. Targets

Herpesviruses have fundamental systems for replication that have been inherited from very ancient ancestors. Current antiherpetic compounds operate against these systems, and there is opportunity for further development of agents in this category. Although a broad specificity might be expected of such agents, thus far this has not proved the case. Certain interactions between conserved proteins have been characterised in some detail (for example, between the catalytic and processivity subunits of HSV-1 DNA polymerase encoded by UL54 and UL42; Zuccola et al., 2000). The specificity of future agents that interfere with such interactions, with regard to the target herpesvirus, will be a matter of interest.

It is also becoming apparent that complex accessory systems, that have arisen more recently in evolution, are essential in enabling the virus to maintain its edge against the host. Therapies against components of these systems are in principle capable of altering this balance and giving the host the advantage, and therefore are worth investigating. Latency is an essential part of the herpesvirus lifestyle, but the genetic systems responsible have been replaced periodically during evolution. Therapies that would break this part of the lifecycle are highly desirable, but appear to be a long way off at present.

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