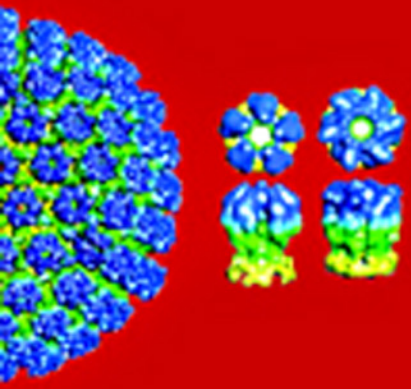
Human Herpesviruses

Biology, Therapy, and Immunoprophylaxis



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Human Herpesviruses

This comprehensive account of the human herpesviruses provides an encyclopedic overview of their basic virology and clinical manifestations. This group of viruses includes human simplex type 1 and 2, Epstein–Barr virus, Kaposi's Sarcoma-associated herpesvirus, cytomegalovirus, HHV6A, 6B, and 7, and varicella-zoster virus. The viral diseases and cancers they cause are significant and often recurrent. Their prevalence in the developed world accounts for a major burden of disease, and as a result there is a great deal of research into the pathophysiology of infection and immunobiology. Another important area covered within this volume concerns antiviral therapy and the development of vaccines. All these aspects are covered in depth, both scientifically and in terms of clinical guidelines for patient care. The text is illustrated generously throughout and is fully referenced to the latest research and developments.

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Preface

Diseases caused by the human herpesviruses were recognized by the earliest practitioners of medicine. Hippocrates, Celsus, Herodotus, Galen, Avicenna and others described cutaneous lesions typical of infections caused by herpes simplex viruses (HSV) 1 and 2, and varicella-zoster virus (VZV). 'Herpes,' the family name of these viruses, is traced to the Greek term for lesions that appeared to creep or crawl over the skin. Among the duties of John Astruc, physician to King Louis XIV, was to understand the diseases of French prostitutes, in Latin, the '*Puellae publicae*', which led to his description of *herpes genitalis*. Distinguishing between genital herpes and syphilis was an obvious concern in this social context as it is now. The modern scientific investigation of HSV can be dated to the work of Gruter, who first isolated the virus and demonstrated its serial transmission in rabbits. During the 19th century, experiments in human subjects showed that HSV and VZV could be transmitted from fluid recovered from HSV and VZV lesions. Demonstrating that Koch's posulates were fulfilled was important but arguably the truly revolutionary discovery about the herpesviruses was made by Andrews and Carmichael in the 1930s who showed that recurrent *herpes labialis* occurred only in adults who already had neutralizing antibodies against HSV. Since our modern understanding of all of the human herpesviruses revolves around latency and reactivation as established facts of their biology, it is important to remember that these concepts are far from obvious and to appreciate the creative insights of Doerr who proposed that recurrent HSV was not an exogenous infection but resulted from stimuli to the cell that caused the endogenous production of a virus-like agent and of Burnet and Williams who perfected the notion that HSV persists for life and "*remains for the most part latent; but under the stimulus of trauma, fever, and so forth it may at any time be called into activity and provoke a visible herpetic lesion.*"

Although their relationships to HSV and VZV were by no means appreciated, the more subtle members of the herpesvirus family began to be discovered after an interval of many hundreds of years. The first of these was human cytomegalovirus (HCMV), which was initially associated with human disease through the detection of enlarged cells containing unusual cytoplasmic inclusions in the urine and organs of infants who were born with signs of intrauterine damage that had been attributed to syphilis. In the early 1950s, HCMV as well as VZV were the first human herpesviruses to be isolated in cultured cells. Within a decade, Epstein-Barr virus (EBV) particles were found in Burkitt's lymphoma cells and EBV was shown to be associated with mononucleosis. By the mid-1990s, three more human herpesviruses, HHV-6A, HHV-6B and HHV-7, which share a tropism for T lymphocytes, were discovered and the etiologic agent of the unusual vascular skin tumor called 'Kaposi's sarcoma, first described in 1872, was identified as "Kaposi's sarcoma-asscoiated herpesvirus (KSHV, HHV8). These four new human herpesviruses were identified during the early years of the human immunodeficiency virus (HIV) epidemic because these viruses caused aggressive disease in HIV-infected patients or were discovered during intensive research on human T cell biology. In each instance, discovery of the human herpesviruses paralleled technologic progress, illustrated by animal models for HSV, cell culture methods for VZV and CMV, the cultivation of B lymphocytes for the detection of EBV and of T lymphocytes for identification of HHV-6 and 7, and differential nucleic acid detection for revealing the existence of HHV8.

Molecular genetics methods demonstrate that the human herpesviruses share a common ancestor. However, each virus has evolved to occupy a particular niche during millions of years of co-evolution with their primate, and eventually human, host. Understanding the nuances of the adaptive strategies that have allowed all of these viruses to be transmitted efficiently and to persist so successfully in the human population, and often in the same individual, constitutes a fascinating enterprise. At the same time, infections caused by these ubiquitous viruses create a substantial global burden of disease affecting healthy and immunocompromised patients and among people living in developed and developing countries. Because of their serious and potentially life-threatening consequences, the human herpesviruses are medically important targets for basic and clinical research.

The goal of this book is to describe the remarkable recent progress towards elucidating the basic and clinical virology of these human pathogens, in conjunction with a summary of the many new insights about their epidemiology, mechanisms of pathogenesis and immune control, approaches to clinical diagnosis and the recognition of the clinical illnesses that result from primary and recurrent herpesvirus infections across the age spectrum. All of the herpesviruses have common genes, structures, replication strategies and mechanisms of defense against the host response but each virus also has unique properties that allow it to find its particular ecological refuge. An unexpected outcome of research over the past decade is the finding that the human herpesviruses have devised many different ways to achieve the same biologic effect, as illustrated by their unique strategies for down-regulation of major histocompatibility complex proteins. Functional similarities exist among these viruses even when they do not share similar genes or infect similar tissues. Each chapter of the book explores these viral themes and variations from the virologic and clinical perspectives. The contributions of the many distinguished authors highlight the basic science aspects of the field, emphasizing the comparative virology of the human herpesviruses and virus-host cell interactions, and the significant clinical developments, including antiviral drugs and vaccines, that are essential for the best practice of medicine in the 21st century. The concluding chapter illustrates how therapies for cancer may emerge from these advances in basic and clinical research, to create a fundamentally new era in the complex history of the relationship between the human herpesviruses and their hosts.

The editors are deeply grateful for the generosity of the authors who have shared their comprehensive knowledge of the human herpesviruses. We hope that this book will serve as a resource for investigators and physicians, and most importantly, that it will motivate a new generation of students and trainees to address the many unresolved questions about these herpesviruses as agents of human disease. Since the genomes of all of these viruses have been sequenced, it is obvious that many genes exist for which functions have not been identified and we now understand that most herpesviral proteins can be expected to have multiple functions. Basic research on the human herpesviruses also reveals fundamental facts about human cellular biology, including surface receptors, metabolic pathways, cell survival mechanisms, malignant transformation as well as innate antiviral defenses. In the clinical realm, every improvement in diagnostic methods expands the spectrum of clinical disorders that are recognized as being caused by these viruses. Clinical interventions exist that could not have been imagined fifty years ago but the need for better therapeutic and preventive measures has become even more apparent as the burden of herpesvirus disease is defined with precision. Given that four human herpesviruses have been discovered in the past 15 years, are there others?

Part I

Introduction: definition and classification of the human herpesviruses

Edited by Bernard Roizman

1

Overview of classification

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Introduction

Taxonomy aims to structure relationships among diverse organisms in order to provide a broader understanding of Nature than is afforded by consideration of organisms in isolation. Since biological systems are shaped by evolution, which is not influenced by the human desire to impose order, any taxonomical scheme is bound to be incomplete and to some extent arbitrary. The criteria applied are necessarily confined to what is technically possible, and thus taxonomy has an important historical component. In addition, taxonomy develops conservatively, since striving for the ideal must be tempered by the need to maintain utility. It is also an unfortunate fact that taxonomy provides fertile soil for debate among a few but is of little interest to most. However, it is beyond dispute that the setting of herpesviruses in a taxonomical framework is vital for understanding the origins and behavior of this fascinating family of organisms.

Historically, herpesvirus taxonomy has been addressed since 1971 by the International Committee on Taxonomy of Viruses (ICTV) (Wildy, 1971). A provisional approach to endowing herpesviruses with formal names (Roizman *et al.*, 1973) was followed by grouping into subfamilies largely on the basis of biological criteria (Roizman *et al.*, 1981). This effort was rather successful, but not free from what turned out in hindsight to be a few misclassifications (Roizman *et al.*, 1992). Further division of the subfamilies into genera utilized molecular data to a greater extent than before, primarily in relation to genome characteristics such as size and structure (Roizman *et al.*, 1992). In the latest report of the ICTV Herpesvirus Study Group (Davison *et al.*, 2005), the family *Herpesviridae* consists of three subfamilies: *Alphaherpesvirinae* (containing the *Simplexvirus*, *Varicellovirus*, *Mardivirus* and *Iltovirus* genera), *Betaherpesvirinae* (containing the *Cytomegalovirus*, *Muromegalovirus* and *Roseolovirus* genera) and *Gammaherpesvirinae* (containing the *Lymphocryptovirus* and *Rhadinovirus* genera). In addition, there is a genus (*Ictalurivirus*) unattached to any subfamily and a large number of species not assigned to genera. The current list is given in Table 1.1. All but one of the viruses assigned to taxa infect mammals or birds, although a substantial number of unassigned herpesviruses have lower vertebrate (reptilian, amphibian and fish) or invertebrate (bivalve) hosts.

Morphological criteria

The primary criterion for inclusion of an agent in the family *Herpesviridae* is that of virion morphology. The virion is spherical, and comprises four major components: the core, the capsid, the tegument and the envelope (see Chapter 3). The diameter of the virion depends on the viral species, but is approximately 200 nm. The core consists of a single copy of a linear, double-stranded DNA molecule packaged at high density into the capsid. The capsid is an icosahedron, and has an external diameter of 125–130 nm. It consists of 162 capsomeres, 12 of which are pentons and 150 hexons, each containing five and six copies, respectively, of the major capsid protein. The capsomeres are joined via the triplexes, each of which contains two copies of one protein and one copy of another. The tegument, which surrounds the capsid, contains perhaps 30 or more viral protein species and is poorly defined structurally. In the tegument, structures positioned with symmetry corresponding to that of the capsid are detectable only in the region close to the capsid. The lipid envelope surrounds the exterior of the tegument, and is studded with at least ten viral membrane

Table 1.1. Herpesvirus taxonomy and nomenclature

Formal name[a]	Abbrev.	Common name[b]	Abbrev.[c]
Subfamily Alphaherpesvirinae			
Genus Simplexvirus			
Ateline herpesvirus 1	AtHV-1	Spider monkey herpesvirus	
Bovine herpesvirus 2	BoHV-2	Bovine mamillitis virus	
Cercopithecine herpesvirus 1	CeHV-1	B-virus	HVB
Cercopithecine herpesvirus 2	CeHV-2	SA8 virus	
Cercopithecine herpesvirus 16	CeHV-16	Herpesvirus papio 2	
Human herpesvirus 1	HHV-1	Herpes simplex virus [type] 1	HSV-1
Human herpesvirus 2	HHV-2	Herpes simplex virus [type] 2	HSV-2
Macropodid herpesvirus 1	MaHV-1	Parma wallaby herpesvirus	
Macropodid herpesvirus 2	MaHV-2	Dorcopsis wallaby herpesvirus	
Saimiriine herpesvirus 1	SaHV-1	Herpesvirus tamarinus	
Genus Varicellovirus			
Bovine herpesvirus 1	BoHV-1	Infectious bovine rhinotracheitis virus	BHV-1
Bovine herpesvirus 5	BoHV-5	Bovine encephalitis virus	BHV-5
Bubaline herpesvirus 1	BuHV-1	Water buffalo herpesvirus	
Canid herpesvirus 1	CaHV-1	Canine herpesvirus	
Caprine herpesvirus 1	CpHV-1	Goat herpesvirus	
Cercopithecine herpesvirus 9	CeHV-9	Simian varicella virus	SVV
Cervid herpesvirus 1	CvHV-1	Red deer herpesvirus	
Cervid herpesvirus 2	CvHV-2	Reindeer herpesvirus	
Equid herpesvirus 1	EHV-1	Equine abortion virus	
Equid herpesvirus 3	EHV-3	Equine coital exanthema virus	
Equid herpesvirus 4	EHV-4	Equine rhinopneumonitis virus	
Equid herpesvirus 8	EHV-8	Asinine herpesvirus 3	
Equid herpesvirus 9	EHV-9	Gazelle herpesvirus	
Felid herpesvirus 1	FeHV-1	Feline rhinotracheitis virus	
Human herpesvirus 3	HHV-3	Varicella-zoster virus	VZV
Phocid herpesvirus 1	PhoHV-1	Harbour seal herpesvirus	
Suid herpesvirus 1	SuHV-1	Pseudorabies virus	PRV
Tentative species in genus Varicellovirus			
Equid herpesvirus 6	EHV-6	Asinine herpesvirus 1	
Genus Mardivirus			
Gallid herpesvirus 2	GaHV-2	Marek's disease virus type 1	MDV-1
Gallid herpesvirus 3	GaHV-3	Marek's disease virus type 2	MDV-2
Meleagrid herpesvirus 1	MeHV-1	Turkey herpesvirus	HVT
Genus Iltovirus			
Gallid herpesvirus 1	GaHV-1	Infectious laryngotracheitis virus	ILTV
Unassigned species in subfamily Alphaherpesvirinae			
Psittacid herpesvirus 1	PsHV-1	Parrot herpesvirus	
Subfamily Betaherpesvirinae			
Genus Cytomegalovirus			
Cercopithecine herpesvirus 5	CeHV-5	African green monkey cytomegalovirus	SCMV
Cercopithecine herpesvirus 8	CeHV-8	Rhesus monkey cytomegalovirus	RhCMV
Human herpesvirus 5	HHV-5	Human cytomegalovirus	HCMV
Pongine herpesvirus 4	PoHV-4	Chimpanzee cytomegalovirus	CCMV
Tentative species in genus Cytomegalovirus			
Aotine herpesvirus 1	AoHV-1	Herpesvirus aotus 1	
Aotine herpesvirus 3	AoHV-3	Herpesvirus aotus 3	

Table 1.1. (*cont.*)

Formal name[a]	Abbrev.	Common name[b]	Abbrev.[c]
Genus Muromegalovirus			
Murid herpesvirus 1	MuHV-1	Mouse cytomegalovirus	MCMV
Murid herpesvirus 2	MuHV-2	Rat cytomegalovirus	RCMV
Genus Roseolovirus			
Human herpesvirus 6	HHV-6		
Human herpesvirus 7	HHV-7		
Unassigned species in subfamily Betaherpesvirinae			
Caviid herpesvirus 2	CavHV-2	Guinea pig cytomegalovirus	GPCMV
Tupaiid herpesvirus 1	TuHV-1	Tree shrew herpesvirus	THV
Subfamily Gammaherpesvirinae			
Genus Lymphocryptovirus			
Callitrichine herpesvirus 3	CalHV-3	Marmoset lymphocryptovirus	Marmoset LCV
Cercopithecine herpesvirus 12	CeHV-12	Herpesvirus papio	
Cercopithecine herpesvirus 14	CeHV-14	African green monkey EBV-like virus	
Cercopithecine herpesvirus 15	CeHV-15	Rhesus lymphocryptovirus	Rhesus LCV
Human herpesvirus 4	HHV-4	Epstein-Barr virus	EBV
Pongine herpesvirus 1	PoHV-1	Herpesvirus pan	
Pongine herpesvirus 2	PoHV-2	Orangutan herpesvirus	
Pongine herpesvirus 3	PoHV-3	Gorilla herpesvirus	
Genus Rhadinovirus			
Alcelaphine herpesvirus 1	AlHV-1	Malignant catarrhal fever virus	AHV-1
Alcelaphine herpesvirus 2	AlHV-2	Hartebeest malignant catarrhal fever virus	
Ateline herpesvirus 2	AtHV-2	Herpesvirus ateles	HVA
Bovine herpesvirus 4	BoHV-4	Movar virus	BHV-4
Cercopithecine herpesvirus 17	CeHV-17	Rhesus rhadinovirus	RRV
Equid herpesvirus 2	EHV-2		
Equid herpesvirus 5	EHV-5		
Equid herpesvirus 7	EHV-7	Asinine herpesvirus 2	
Hippotragine herpesvirus 1	HiHV-1	Roan antelope herpesvirus	
Human herpesvirus 8	HHV-8	Kaposi's sarcoma-associated herpesvirus	KSHV
Murid herpesvirus 4	MuHV-4	Murine gammaherpesvirus 68	MHV-68
Mustelid herpesvirus 1	MusHV-1	Badger herpesvirus	
Ovine herpesvirus 2	OvHV-2	Sheep-associated malignant catarrhal fever virus	
Saimiriine herpesvirus 2	SaHV-2	Herpesvirus saimiri	HVS
Tentative species in genus Rhadinovirus			
Leporid herpesvirus 1	LeHV-1	Cottontail rabbit herpesvirus	
Leporid herpesvirus 2	LeHV-2	Herpesvirus cuniculi	
Leporid herpesvirus 3	LeHV-3	Herpesvirus sylvilagus	
Marmodid herpesvirus 1	MarHV-1	Woodchuck herpesvirus	
Unassigned species in subfamily Gammaherpesvirinae			
Callitrichine herpesvirus 1	CalHV-1	Herpesvirus saguinus	
Unassigned genus Ictalurivirus* in family *Herpesviridae			
Ictalurid herpesvirus 1	IcHV-1	Channel catfish virus	CCV
Unassigned viruses in family Herpesviridae			
Acipenserid herpesvirus 1	AciHV-1	White sturgeon herpesvirus 1	
Acipenserid herpesvirus 2	AciHV-2	White sturgeon herpesvirus 2	
Acciptrid herpesvirus 1	AcHV-1	Bald eagle herpesvirus	
Anatid herpesvirus 1	AnHV-1	Duck plague herpesvirus	
Anguillid herpesvirus 1	AngHV-1	Japanese eel herpesvirus	

(*cont.*)

Table 1.1. (*cont.*)

Formal name[a]	Abbrev.	Common name[b]	Abbrev.[c]
Ateline herpesvirus 3	AtHV-3	Herpesvirus ateles strain 73	
Boid herpesvirus 1	BoiHV-1	Boa herpesvirus	
Callitrichine herpesvirus 2	CalHV-2	Marmoset cytomegalovirus	
Caviid herpesvirus 1	CavHV-1	Guinea pig herpesvirus	
Caviid herpesvirus 3	CavHV-3	Guinea pig herpesvirus 3	
Cebine herpesvirus 1	CbHV-1	Capuchin herpesvirus AL-5	
Cebine herpesvirus 2	CbHV-2	Capuchin herpesvirus AP-18	
Cercopithecine herpesvirus 3	CeHV-3	SA6 virus	
Cercopithecine herpesvirus 4	CeHV-4	SA15 virus	
Cercopithecine herpesvirus 10	CeHV-10	Rhesus leukocyte-associated herpesvirus strain 1	
Cercopithecine herpesvirus 13	CeHV-13	Herpesvirus cyclopis	
Chelonid herpesvirus 1	ChHV-1	Grey patch disease of turtles	
Chelonid herpesvirus 2	ChHV-2	Pacific pond turtle herpesvirus	
Chelonid herpesvirus 3	ChHV-3	Painted turtle herpesvirus	
Chelonid herpesvirus 4	ChHV-4	Argentine turtle herpesvirus	
Ciconiid herpesvirus 1	CiHV-1	Black stork herpesvirus	
Columbid herpesvirus 1	CoHV-1	Pigeon herpesvirus	
Cricetid herpesvirus	CrHV-1	Hamster herpesvirus	
Cyprinid herpesvirus 1	CyHV-1	Carp pox herpesvirus	
Cyprinid herpesvirus 2	CyHV-2	Goldfish herpesvirus	
Elapid herpesvirus 1	EpHV-1	Indian cobra herpesvirus	
Elephantid herpesvirus 1	ElHV-1	Elephant [loxodontal] herpesvirus	
Erinaceid herpesvirus 1	ErHV-1	European hedgehog herpesvirus	
Esocid herpesvirus 1	EsHV-1	Northern pike herpesvirus	
Falconid herpesvirus 1	FaHV-1	Falcon inclusion body diseases	
Gruid herpesvirus 1	GrHV-1	Crane herpesvirus	
Iguanid herpesvirus 1	IgHV-1	Green iguana herpesvirus	
Lacertid herpesvirus	LaHV-1	Green lizard herpesvirus	
Lorisine herpesvirus 1	LoHV-1	Kinkajou herpesvirus	
Murid herpesvirus 3	MuHV-3	Mouse thymic herpesvirus	
Murid herpesvirus 5	MuHV-5	Field mouse herpesvirus	
Murid herpesvirus 6	MuHV-6	Sand rat nuclear inclusion agents	
Ostreid herpesvirus 1	OsHV-1	Pacific oyster herpesvirus	OHV
Ovine herpesvirus 1	OvHV-1	Sheep pulmonary adenomatosis-associated herpesvirus	
Percid herpesvirus 1	PeHV-1	Walleye epidermal hyperplasia	
Perdicid herpesvirus 1	PdHV-1	Bobwhite quail herpesvirus	
Phalacrocoracid herpesvirus 1	PhHV-1	Cormorant herpesvirus	
Pleuronectid herpesvirus 1	PlHV-1	Turbot herpesvirus	
Ranid herpesvirus 1	RaHV-1	Lucké frog herpesvirus	
Ranid herpesvirus 2	RaHV-2	Frog herpesvirus 4	
Salmonid herpesvirus 1	SalHV-1	Herpesvirus salmonis	
Salmonid herpesvirus 2	SalHV-2	*Oncorhynchus masou* herpesvirus	
Sciurid herpesvirus 1	ScHV-2	European ground squirrel cytomegalovirus	
Sciurid herpesvirus 2	ScHV-2	American ground squirrel cytomegalovirus	
Sphenicid herpesvirus 1	SpHV-1	Black footed penguin herpesvirus	
Strigid herpesvirus 1	StHV-1	Owl hepatosplenitis herpesvirus	
Suid herpesvirus 2	SuHV-2	Swine cytomegalovirus	

[a] Type species of genera are in italics.
[b] Some viruses have several common names. Only one is given for each.
[c] The list is restricted to abbreviations used in this publication.
Adapted from Davison *et al.* (2005).

glycoproteins, in addition to some cellular proteins. The protein composition of the tegument and envelope varies widely across the family.

Serological criteria

In contrast to virion morphology, which operates as a criterion at the level of the family, serological relationships are useful only for detecting closely related viruses. Neutralizing antibodies form a subset of serological tools, and are directed against some of the envelope glycoproteins.

Biological criteria

The observation that several distinct herpesviruses have been found in the most extensively studied animals implies that the number of herpesvirus species in Nature must far exceed that catalogued to date. The natural host range of individual viruses is usually restricted to a single species. Occasional transfer to other species can occur, although it could be argued that the settings involved (farms, zoos and keeping pets) are the results of human activities. In experimental animal systems, some members of the *Alphaherpesvirinae* can infect a wide variety of species, whereas *Beta-* and *Gammaherpesvirinae* are very restricted. The same general observation characterizes growth in cell culture.

Herpesviruses are highly adapted to their hosts, and severe symptoms of infection are usually limited to very young or immunosuppressed individuals. Natural transmission routes range from aerosol spread to mucosal contact. Most herpesviruses establish a systemic infection associated with a cell-associated viraemia, although infection with some members of genus *Simplexvirus* is limited to the epithelium at the inoculation site and to innervating sensory neurons. Herpesviruses have elaborate means of modulating the host responses to infection, and are able to establish lifelong latent infections. In simplified, general terms, the cell types involved in latency are the neuron for the *Alphaherpesvirinae*, the monocyte lineage for the *Betaherpesvirinae*, and lymphocytes for the *Gammaherpesvirinae*.

Genomic criteria

Herpesvirus genomes studied to date range in size from about 125 to 240 kbp, and the most extensively characterized contain from about 70 to 165 genes. Prior to the generation of extensive sequence data, genome structures (see Chapter 2) were an aid to classification. However, the usefulness of this criterion is limited, since similar structures have evidently evolved more than once in the family. Nucleic acid hybridization data also provided input and, like serological data, are limited to demonstrating relationships between closely related viruses. As with other groups of organisms, data derived from nucleotide and amino acid sequences have gained increasing prominence and now dominate herpesvirus taxonomy. Figure 1.1 shows one example of such data, a phylogenetic tree based upon amino acid sequence alignments (McGeoch *et al.*, 1994, 1995, 2000). Another approach yielded different schemes of relationships, but was based on analytical criteria not widely accepted in depicting evolutionary relationships (Karlin *et al.*, 1994).

It has long been thought from the apparent adaptation of herpesviruses to their hosts that a substantial degree of co-evolution has occurred. Similarities between the phylogenetic relationships among the viruses and those among their hosts provide strong support for this model, and in some instances indicate that co-speciation has occurred. A number of possible exceptions have been noted and are discussed in further detail in Chapter 2.

Species definition

A virus species is defined as a polythetic class of viruses constituting a replicating lineage and occupying a particular ecological niche (Van Regenmortel, 1989, 1990). Members of a polythetic class share a subset of properties, with each property possessed by several members but no property possessed by all. Herpesviruses are defined as separate species if their nucleotide sequences differ in a readily assayable and distinctive manner across the entire genome and if they occupy different ecological niches by virtue of their distinct epidemiology and pathogenesis or their distinct natural hosts (Roizman *et al.*, 1992; Roizman and Pellett, 2001; Davison *et al.*, 2005). However, genomic data have come to dominate biological properties, with taxa corresponding to genetic lineages defined by sequence comparisons and identification of genes unique to certain lineages. An increasing number of herpesviruses in the tissues of various animals are being inferred from short PCR-derived sequences, usually from a single locus in the genome and often in the absence of any other information. These "virtual viruses" cannot readily be classified under the current species definition. However, their incorporation (perhaps in a special category) could be facilitated by

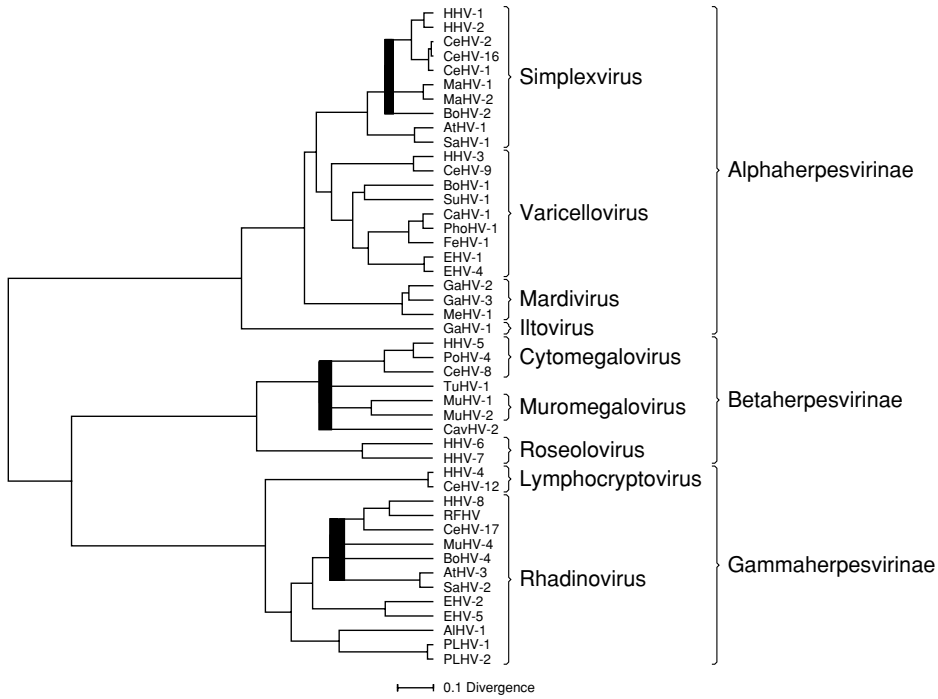

Fig. 1.1. Composite phylogenetic tree for herpesviruses. The tree is based on amino acid sequence alignments of eight sets of homologous genes, constructed from maximum-likelihood trees for subsets of these genes, with molecular clock imposed. Thick lines designate regions of uncertain branching. Formal species abbreviations and designations for genera and subfamilies are given on the right (see Table 1.1). Viruses that are not yet incorporated formally into genera are denoted in italics. Three unclassified viruses are included (RFHV, retroperitoneal fibromatosis herpesvirus of macaques; PLHV-1 and PLHV-2, porcine lymphotropic herpesviruses 1 and 2). Modified from McGeoch *et al.* (2000) with permission from the American Society for Microbiology.

addition to the species definition of a third criterion, that of phylogeny based on the relatedness of conserved genes. Recognition that taxonomy should reflect evolutionary history would also aid rational incorporation of herpesviruses of lower vertebrates and invertebrates into a taxonomy that is currently dominated by herpesviruses of higher vertebrates. Current problems in this area, and a suggested solution, are given in Chapter 2.

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2

Comparative analysis of the genomes

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Introduction

Members of the family *Herpesviridae* replicate their genomes in the infected cell nucleus and have a characteristic virion morphology, which consists of the envelope, tegument, capsid and core (Davison and Clements, 1997). An extensive description of virion structure is given in Chapter 3. The present chapter focuses on the viral genome, which occupies the core of the virus particle. Electron microscopy of negatively stained capsids gives the impression that the core consists of the viral DNA molecule wrapped toroidally around a protein spindle (Furlong *et al.*, 1972). Images reconstructed from electron micrographs of virions frozen in ice in the absence of stain, a technique by which morphology is better preserved, show that the core consists of the DNA packed at high density in liquid crystalline form, probably as a spool lacking a spindle (Booy *et al.*, 1991; Zhou *et al.*, 1999).

Herpesvirus genomes consist of linear, double-stranded DNA molecules that range in size from about 125 to 240 kbp and in nucleotide composition from 32 to 75% G+C, depending on the virus species (Honess, 1984). The genome termini are not covalently closed (as in the *Poxviridae;* Moss, 2001) or covalently linked to a protein (as in the *Adenoviridae*; Shenk, 2001). In those herpesvirus genomes that have been examined in sufficient detail, unpaired nucleotides are present at the termini; for example, HSV-1, VZV and HCMV have a single 3′-overhanging nucleotide at each terminus (Mocarski and Roizman, 1982; Davison, 1984; Tamashiro and Spector, 1986). Larger herpesvirus genomes are accommodated in larger capsids, but the relationship is not proportional, as the packing density of the DNA varies somewhat between species (Trus *et al.*, 1999; Bhella *et al.*, 2000). The reasons for the striking range in nucleotide composition of herpesvirus genomes are not clear, but a similar phenomenon is found in other virus families and in cellular organisms. In contrast to the *Alpha-* and *Betaherpesvirinae*, the genomes of most *Gammaherpesvirinae* are generally deficient in the CG dinucleotide (Table 2.1). In vertebrate genomes, this phenomenon is thought to be due to spontaneous deamination of 5-methylcytosine residues in DNA to thymidine residues, followed by fixation through DNA replication. CG depletion in herpesviruses, and concomitant enrichment in TG and CA, has been taken as indicative of latency in dividing cell populations, in which the latent genome is obliged to replicate as host cells divide (Honess *et al.*, 1989). Thus, HSV-1, which is resident in non-dividing neurons, has a CG content consistent with its nucleotide composition, whereas EBV, which latently infects dividing B cell populations, is depleted. Local CG suppression of the major immediate early gene locus of HCMV has also been noted (Honess *et al.*, 1989).

Genome structures

Herpesvirus genomes are not simple lengths of unique DNA, but characteristically contain direct or inverted repeats. The reasons for this are not known, but it is intriguing that similar structures appear to have arisen independently on several occasions during herpesvirus evolution. Herpesvirus genomes are thought to replicate by circularization, followed by production of concatemers and cleavage of unit-length genomes during packaging into capsids (Boehmer and Lehman, 1997). The explanation for the presence of repeats is probably connected in some way with the mode of DNA replication, rather than with any advantage gained by having multiple copies of certain genes. Although greater expression would be a consequence of repeated genes, this appears a simplistic explanation in an evolutionary context, since subtler processes of nucleotide

Table 2.1. Sequenced herpesvirus genomes

Common name	Strain[a]	Abbreviation		Accession	Size (bp)[b]	Composition[c]		Reference
		Common	Formal			G+C	CG	
Mammalian herpesvirus group[d]								
Alphaherpesvirinae								
Simplexvirus								
Herpes simplex virus type 1	17	HSV-1	HHV-1	X14112	152261	68.3	1.01	McGeoch *et al.* (1988)
Herpes simplex virus type 2	HG52	HSV-2	HHV-2	Z86099	154746	70.4	1.06	Dolan *et al.* (1998)
B virus	E2490	HVB	CeHV-1	AF533768	156789	74.5	1.09	Perelygina *et al.* (2003)
SA8	B264	SA8	CeHV-2	AY714813	150715	76.0	1.09	Tyler *et al.* (2005)
Herpes papio 2	X313	HVP2	CeHV-16	DQ149153	156487	76.1	1.08	Tyler and Severini (2006)
Varicellovirus								
Varicella-zoster virus	Dumas	VZV	HHV-3	X04370	124884	46.0	1.14	Davison and Scott (1986)
	Oka vaccine			AB097932	125078			Gomi *et al.* (2002)
	Oka parental			AB097933	125125			Gomi *et al.* (2002)
	MSP			AY548170	124883			Grose *et al.* (2004)
	BC			AY548171	125459			Grose *et al.* (2004)
	Varilrix			DQ008354	124821			Vassilev (2005)
	Varilrix			DQ008355	124815			Vassilev (2005)
	HJO			AJ871403	124928			Fickenscher *et al.* (unpublished)
Simian varicella virus	Delta	SVV	CeHV-9	AF275348	124138	40.4	1.12	Gray *et al.* (2001)
Bovine herpesvirus 1	[Cooper]	BHV-1	BoHV-1	AJ004801	135301	72.4	1.19	Schwyzer & Ackermann (1996)
Bovine herpesvirus 5	SSV507/99	BHV-5	BoHV-5	AY261359	138390	74.9	1.17	Delhon *et al.* (2003)
Pseudorabies virus	[Kaplan]	PRV	SuHV-1	BK001744	143461	73.6	1.12	Klupp *et al.* (2004)
Equine herpesvirus 1	Ab4	EHV-1	EHV-1	AY665713	150224	56.7	0.99	Telford *et al.* (1992)
	V592			AY464052	149430			Nugent *et al.* (2006)
Equine herpesvirus 4	NS80567	EHV-4	EHV-4	AF030027	145597	50.5	0.93	Telford *et al.* (1998)
Mardivirus								
Marek's disease virus type 1	Md5	MDV-1	GaHV-2	AF243438	177874	44.1	1.01	Tulman *et al.* (2000)
	GA			AF147806	174077			Lee *et al.* (2000)
	Md11			[AY510475]	170950			Niikura *et al.* (unpublished)
Marek's disease virus type 2	HPRS24	MDV-2	GaHV-3	AB049735	164270	53.6	1.23	Izumiya *et al.* (2001)
Turkey herpesvirus	FC126	HVT	MeHV-1	AF291866	159160	47.6	1.11	Afonso *et al.* (2001)
Iltovirus								
Infections laryngotracheitis virus	[SA-2]	ILTV	GaHV-1	NC006623	148687	48.2	1.01	Thureen and Keeler (2006)
Psittacid herpesvirus 1[e]	97-0001	PsHV-1	PsHV-1	AY372243	163025	60.9	1.21	Thureen and Keeler (2006)
Betaherpesvirinae								
Cytomegalovirus								
Human cytomegalovirus	Merlin	HCMV	HHV-5	AY446894	235645	57.5	1.19	Dolan *et al.* (2004)
	AD169			X17403	229354			Chee *et al.* (1990)
	AD169			BK000394	230287			Davison *et al.*(2003a)
	AD169			[AC146999]	[233739]			Murphy *et al.* (2003b)
	Towne			[AY315197]	[231236]			Dunn *et al.* (2003)
	Towne			[AC146851]	[229483]			Murphy *et al.* (2003b)
	Toledo			[AC146905]	[226889]			Murphy *et al.* (2003b)
	PH			[AC146904]	[229700]			Murphy *et al.* (2003b)
	TR			[AC146906]	[234881]			Murphy *et al.* (2003b)
	FIX			[AC146907]	[229209]			Murphy *et al.* (2003b)
Chimpanzee cytomegalovirus	–	CCMV	PoHV-4	AF480884	241087	61.7	1.11	Davison *et al.* (2003a)
Rhesus cytomegalovirus	68-1	RhCMV	CeHV-8	AY186194	221454	49.1	0.99	Hansen *et al.* (2003)
Unassigned								
Tupaiid herpesvirus	2	THV	TuHV-1	AF281817	195859	66.6	1.28	Bahr & Darai (2001)
Muromegalovirus								
Murine cytomegalovirus	Smith	MCMV	MuHV-1	U68299	230278	58.7	1.22	Rawlinson *et al.* (1996)
Rat cytomegalovirus	Maastricht	RCMV	MuHV-2	AF232689	230138	61.0	1.25	Vink *et al.* (2000)
Roseolovirus								
Human herpesvirus 6	U1102	HHV-6A	HHV-6	X83413	159321	42.4	1.13	Gompels *et al.* (1995)
	Z29	HHV-6B		AF157706	162114	42.8	1.10	Dominguez *et al.* (1999)
	HST			AB021506	161573			Isegawa *et al.* (1999)

(*cont.*)

Table 2.1. (*cont.*)

Common name	Strain[a]	Abbreviation		Accession	Size (bp)[b]	Composition[c]		Reference
		Common	Formal			G+C	CG	
Human herpesvirus 7	JI	HHV-7	HHV-7	U43400	144861	35.3	0.80	Nicholas (1996)
	RK			AF037218	153080			Megaw *et al.* (1998)
Gammaherpesvirinae								
Lymphocryptovirus								
Epstein–Barr virus	[B95-8]	EBV	HHV-4	AJ507799	171823	59.5	0.61	de Jesus *et al.* (2003)
	GD1			AY961628	171656			Zeng *et al.* (2005)
	AG876			DQ279927	172764			Dolan *et al.* (2006)
	B95-8			V01555	172281			Baer *et al.* (1984)
Marmoset lymphocryptovirus	CJ0149	marmoset LCV	CalHV-3	AF319782	149696	49.3	0.70	Rivailler *et al.* (2002a)
Rhesus lymphocryptovirus	LCL8664	rhesus LCV	CeHV-15	AY037858	171096	61.9	0.68	Rivailler *et al.* (2002b)
Rhadinovirus								
Human herpesvirus 8	BC-1	HHV-8	HHV-8	U75698	[137508]	53.5	0.81	
				U75699	801	84.5	0.92	Russo *et al.* (1996)
	–			U93872	[133661]			Neipel *et al.* (1997)
Rhesus rhadinovirus	17577	RRV	CeHV-17	AF083501	133719	52.2	1.11	Searles *et al.* (1999)
	26-95			AF210726	130733			Alexander *et al.* (2000)
Murine herpesvirus 68[f]	WUMS	MHV-68	MuHV-4	U97553	119450	47.2	0.43	Virgin *et al.* (1997)
	g2.4			AF105037	119550			Nash *et al.* (2001)
Bovine herpesvirus 4	66-p-347	BHV-4	BoHV-4	AF318573	108873	41.4	0.23	Zimmermann *et al.* (2001)
				AF092919	2267	71.2	0.42	
Herpesvirus ateles	73	HVA	AtHV-3	AF083424	108409	36.6	0.40	Albrecht (2000)
				AF126541	1582	77.1	0.79	
Herpesvirus saimiri	A11	HVS	SaHV-2	X64346	112930	34.5	0.33	Albrecht *et al.* (1992)
				K03361	1444	70.9	0.61	
	C488			AJ410493	113027			Ensser *et al.* (2003)
				AJ410494	1458			
Equine herpesvirus 2	86/67	EHV-2	EHV-2	U20824	184427	57.5	0.63	Telford *et al.* (1995)
Alcelaphine herpesvirus 1	C500	AHV-1	AlHV-1	AF005370	130608	46.2	0.42	Ensser *et al.* (1997)
				AF005368	1113	72.0	0.69	
Ovine herpesvirus 2	BJ1035	OvHV-2	OvHV-2	AY839756	135135	52.9	0.58	Stewart *et al.* (unpublished)
Fish herpesvirus group[d]								
Undefined subfamily								
Ictalurivirus								
Channel catfish virus	Auburn 1	CCV	IcHV-1	M75136	134226	56.2	1.1	Davison (1992)
Bivalve herpesvirus group[d]								
Undefined subfamily								
Undefined genus								
Ostreid herpesvirus 1	–	OHV	OsHV-1	AY509253	207439	38.7	0.68	Davison *et al.* (2005)

[a] Square brackets indicate the strain that was used most extensively in assembling a sequence combining data from several strains. A hyphen indicates that the strain was not specified.

[b] Sizes were obtained from the latest version of the accessions, and may differ from those in the references through correction of errors. Square brackets indicate sequences that fall marginally short of full length: for MDV-1 and HCMV strains, sequences are for bacterial artificial chromosomes, the sizes representing a deleted form of the genome plus the vector; for HHV-8 strains, the sequence at the right end of the unique region was not determined. For members of subfamily *Gammaherpesvirinae* other than EHV-2, actual genome sizes are larger than those listed (approximately 150–180 kbp), owing to the presence of variable copy numbers of terminal repeats at both ends of the genome. Where a single value is given, this represents either the unique region flanked by partial terminal repeats or the unique region only. Where two values are given, the first is for the unique region and the second is for the terminal repeat.

[c] G + C content is given as moles %, CG content is given as observed/expected frequency, taking into account overall nucleotide composition. Where multiple strains have been sequenced for a species, values are given for one strain only. For members of the *Gammaherpesvirinae*, values are given for the deposited sequences, which consist either of the unique region flanked by partial terminal repeat sequence, the unique region only, or separate accessions for the unique region and terminal repeat.

[d] This taxon is used for the purposes of discussion, and, unlike the others in the Table, has no formal standing.

[e] PsHV-1 is the closest relative of ILTV and is placed informally in this genus.

[f] MHV-68 strains g2.4 and WUMS are essentially identical, since the latter was derived from the former.

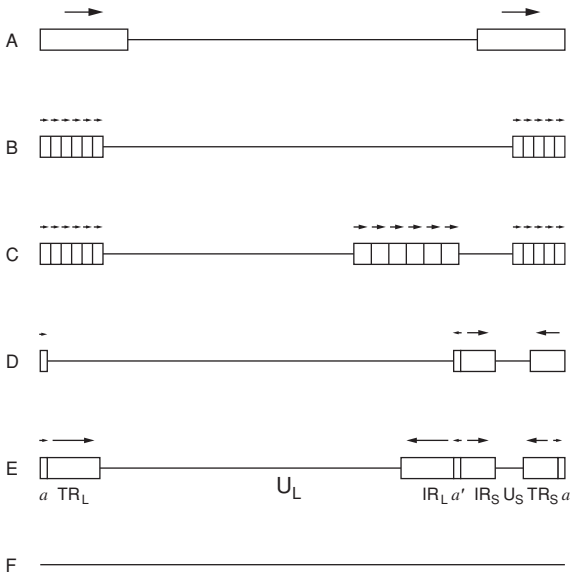

Fig. 2.1. Classes of herpesvirus genome structures (not to scale) as defined by Roizman and Pellett (2001). Unique and repeat regions are shown as horizontal lines and rectangles, respectively. The orientations of repeats are shown by arrows. The nomenclature of unique and repeat regions, including the terminal redundancy (*a*) and its internal, inverted copy (*a*′), is indicated for the class E genome.

substitution can readily alter transcriptional levels over a much greater range. In addition, repeats often do not contain protein-coding regions. As elaborated below, certain genomes exhibit a further structural complexity known as segment inversion, in which unique regions flanked by inverted repeats are found in both orientations in virion DNA. Thus, a genome with two such unique regions would produce either two or four isomers depending on whether one or both regions invert. This phenomenon is probably a consequence of recombination between repeats in concatemeric DNA. Isomers are functionally equivalent (Jenkins and Roizman, 1986), and segment inversion appears to be unrelated to the biology of the virus.

Figure 2.1 shows the major classes of genome structure found among the herpesviruses, as summarized by Roizman and Knipe (2001). The class A genome consists of a unique sequence flanked by a direct repeat. It was first described for CCV (Chousterman *et al.*, 1979), but is also represented among the *Betaherpesvirinae* (HHV-6: Lindquester and Pellett, 1991; Martin *et al.*, 1991; HHV-7: Dominguez *et al.*, 1996; Ruvolo *et al.*, 1996) and one member of the *Gammaherpesvirinae* (EHV-2; Browning and Studdert, 1989). In these examples, the direct repeat is several kbp in size. Other members of the *Betaherpesvirinae* also have this arrangement, but the repeat is smaller, at 504 bp in RCMV (Vink *et al.*, 1996) and 30–31 bp in MCMV (Marks and Spector, 1988; Rawlinson *et al.*, 1996).

Class B genomes also have directly repeated sequences at the termini, but these consist of variable copy numbers of a tandemly repeated sequence of 0.8–2.3 kbp. This arrangement characterizes most *Gammaherpesvirinae* in the *Rhadinovirus* genus, such as HVS and HHV-8 (Bornkamm *et al.*, 1976; Russo *et al.*, 1996). The repeated regions may comprise up to 30% of the DNA molecule (Bornkamm *et al.*, 1976; Lagunoff and Ganem, 1997). The presence of additional terminal repeat sequences in inverse orientation internally in the genome gives rise to a related structure, which is present in another member of the *Gammaherpesvirinae*, cottontail rabbit herpesvirus (Cebrian *et al.*, 1989). The virion DNA of this virus exhibits segment inversion because the two unique regions are flanked by inverted repeats. The class C structure represents another derivative of class B, in which an internal set of direct repeats is present but is unrelated to the terminal set. EBV, a member of the *Gammaherpesvirinae* in the *Lymphocryptovirus* genus, has this arrangement (Given and Kieff, 1979). Segment inversion does not occur because the internal and terminal repeats are not related.

Class D genomes contain two unique regions (U_L and U_S), each flanked by inverted repeats (TR_L/IR_L and TR_S/IR_S). This structure is characteristic of *Alphaherpesvirinae* in the *Varicellovirus* genus, such as PRV and VZV (Rixon and Ben-Porat, 1979; Dumas *et al.*, 1981), and has also evolved separately in salmonid herpesvirus 1 (Davison, 1998). Segment inversion occurs inasmuch as equimolar amounts of genomes containing the two orientations of U_S are found in virion DNA, but U_L is present predominantly or completely in one orientation. The latter feature cannot be explained solely by recombination, and is probably due also to the presence of the cleavage signal solely or largely in the region comprising TR_L/IR_L and one end of U_L (Davison, 1984; Rall *et al.*, 1991).

Class E is the most complex genome structure, and was the first to be described, for HSV-1 (Sheldrick and Berthelot, 1975). It is similar to class D, except that TR_L/IR_L is much larger and segment inversion gives rise to four equimolar genome isomers (Wadsworth *et al.*, 1975; Hayward *et al.*, 1975; Delius and Clements, 1976; Clements *et al.*, 1976; Wilkie and Cortini, 1976). Also, class E genomes are terminally redundant, containing a sequence of a few hundred bp (termed the *a* sequence) that is repeated directly at the genome termini and inversely at the IR_L-IR_S junction (Sheldrick and Berthelot, 1975; Grafstrom *et al.*, 1975a,b; Wadsworth *et al.*, 1976; Hyman *et al.*, 1976).

Minor proportions of genomes contain multiple copies of the *a* sequence at the left terminus or the IR_L-IR_S junction (Wilkie, 1976; Wagner and Summers, 1978; Locker and Frenkel, 1979). The class E arrangement is characteristic of *Alphaherpesvirinae* in the *Simplexvirus* genus, and has evolved independently in the lineage giving rise to HCMV and CCMV, members of the *Betaherpesvirinae* (Weststrate *et al.*, 1980; DeMarchi, 1981; Davison *et al.*, 2003a). A structure similar to both class D and E genomes has also evolved in an invertebrate herpesvirus, OsHV-1 (Davison *et al.*, 2005). This contains two segments, each consisting of a unique region flanked by an substantial inverted repeat, linked via an additional small, non-inverting unique region. As in class E genomes, the two segments undergo inversion, but, like class D, the genome is not terminally redundant.

Class F is represented by a member of the *Betaherpesvirinae*, THV, which apparently lacks the types of inverted and direct repeats that characterize other herpesvirus genomes (Koch *et al.*, 1985; Albrecht *et al.*, 1985). However, since the genome ends of THV have not been analyzed directly, the existence of this unusual structure is considered tentative.

Genome sequences

Table 2.1 lists the 39 herpesvirus species for which genome sequences are currently available in the public databases. Additional strains have been sequenced for some species, yielding a total of 63 sequenced strains. The ease of generating data will continue to expand the number of herpesvirus species and strains sequenced in coming years. Indeed, substantial inroads have been made into large-scale studies of strain variation for certain of the human herpesviruses. It appears that the scale and extent of variation is lineage dependent, with *Betaherpesvirinae* more variable than *Gammaherpesvirinae*, and *Alphaherpesvirinae* the least variable (Murphy *et al.*, 2003b; Dolan *et al.*, 2004; Poole *et al.*, 1999; Midgley *et al.*, 2003; Muir *et al.*, 2002; Gomi *et al.*, 2002). The development of tools to study variation in increasing detail will enhance understanding of viral epidemiology, in terms both of its relation to human evolution and migration and of the changes that are occurring in human populations at the present time.

Gene content

Sequencing herpesvirus genomes is now routine, but the process of describing gene content (annotation) is not trivial. Thus, as with other groups of organisms, the quality of annotation of herpesvirus entries in the public databases varies widely. It is an unfortunate fact that no set of objective criteria is sufficient to interpret the gene content of a sequence completely. Although most genes can be catalogued relatively easily, there are genuine difficulties in identifying all of them, even in the best characterized herpesviruses.

A primary criterion in defining gene content involves identifying open reading frames (ORFs), usually those initiated by methionine (ATG) codons. A tendency to include ORFs that do not encode proteins may be reduced by setting a minimum size. Comparative genomics, which operates on the principle that genes are conserved in evolution, and algorithms that compare sequence patterns within ORFs to the protein-coding regions of known genes, are also useful. However, these tools yield results with least confidence when applied to small, spliced, overlapping or poorly conserved ORFs, and in instances where translation initiates from internal codons, alternative splicing occurs, or esoteric translational mechanisms are employed (e.g., suppression of termination codons and forms of translational editing). In addition to sequence analysis, experimental data on production of an RNA or protein from an ORF provides important imput, although even this falls short of proving functionality. Also, most approaches are aimed at identifying protein-coding genes, and cannot detect genes that encode functional transcripts that are not mRNAs.

The use of different criteria for gene identification may create a degree of uncertainty and debate, and lead to different pictures of gene layout. The case of HCMV provides a contemporary example. In the first analysis of the gene content of HCMV strain AD169, Chee *et al.* (1990) catalogued 189 protein-coding ORFs (counting duplicates once only). Later, the gene number was reduced to 147 by comparing the HCMV and CCMV genomes, allowing, where appropriate, for the presence of genes unique to either genome (Davison *et al.*, 2003a,b). As modified criteria were applied, this number rebounded in a series of increments, first to 157 (Yu *et al.*, 2003), next to 171 (Murphy *et al.*, 2003a), then to 220 (Murphy *et al.*, 2003b) and finally to 232 (Varnum *et al.*, 2004). Although the conservative numbers in this example are more supportable, the existence of unrecognized genes should not be ruled out even in well-characterized genomes, and candidates should be examined rigorously. For example, new genes were identified in previously analysed sequences for VZV (Kemble *et al.*, 2000) and HHV-8 (Glenn *et al.*, 1999).

The genes of HSV-1, presumably like those of all herpesviruses, are transcribed by host RNA polymerase II

(Wagner, 1985; Roizman and Knipe, 2001). Transcription of the first genes to be expressed, the immediate early genes, does not require ongoing protein synthesis, and is enhanced by a tegument protein at low multiplicities of infection (O'Hare, 1993). Some of the immediate early proteins regulate expression of early and late genes (Honess and Roizman, 1974). Early genes, defined as those expressed in the presence of immediate early proteins and before the onset of DNA replication, include enzymes involved in nucleotide metabolism and DNA replication and a number of envelope glycoproteins. Some late genes are expressed at low levels under early conditions, but full expression of "leaky" and "true" late genes is dependent on DNA replication; these genes encode mainly virion proteins. Although the details differ, a similar pattern of regulated gene expression is characteristic of all herpesviruses examined; for example, HCMV (Stinski, 1978), HHV-8 (Sarid *et al.*, 1998) and CCV (Silverstein *et al.*, 1995). In addition, herpesviruses express RNAs whose functions apparently do not involve translation. The best characterized are small RNAs probably transcribed by RNA polymerase III in *Gammaherpesvirinae* such as EBV (Rosa *et al.*, 1981) and MHV-68 (Bowden *et al.*, 1997). Larger noncoding RNAs transcribed by RNA polymerase II include the latency-associated transcripts in HSV-1 (Stevens *et al.*, 1987) and several virion-associated RNAs in HCMV (Bresnahan and Shenk, 2000).

With the exception of a small number of genes that are expressed by splicing from a common 5′-leader, such as the EBNA genes of EBV (Bodescot *et al.*, 1987) and the IE1 and IE2 genes of HCMV (Stenberg *et al.*, 1985), herpesvirus genes have individual promoters. However, it is common for genes to share a polyadenylation site, leading to families of 3′-coterminal transcripts (Wagner, 1985). Apart from families of duplicated genes, there is no pronounced clustering of genes on the basis of function or kinetics of expression. Splicing is uncommon throughout the family, affecting no more than about 20% of the gene number in any genome. Most splicing involves genes that are relatively recent evolutionary developments, and *Beta-* and *Gammaherpesvirinae* have more spliced genes than *Alphaherpesvirinae*.

Genome comparisons and evolution

The availability of extensive sequence data for herpesviruses has facilitated detailed phylogenetic analyses of the family based on amino acid sequence comparisons of conserved genes, as described in Chapter 1. In this section, an overview is given of genetic relatedness at selected levels in the phylogenetic tree, starting with the three major groups that encompass all known herpesviruses, proceeding to the best characterized of these groups, and ending with one subfamily in this group. In chronological terms, this proceeds from earlier to more recent evolutionary events. Detailed information on the gene content of, and the relationships between, the human herpesviruses is available elsewhere in this book.

Three major groups

Three major groups of viruses possess the herpesvirus morphology, including closely similar capsid structures, but share very little genetic similarity (Davison, 1992; Booy *et al.*, 1996; Davison *et al.*, 2005). Viruses in the best characterized group infect mammals, birds and reptiles, viruses in the second group infect amphibians and fish, and the third group contains the single known herpesvirus of an invertebrate, the oyster. Currently, the family *Herpesviridae* comprises the first group classified into three subfamilies and component genera, one member (CCV) of the second group representing an unassigned genus, and the oyster virus is a floating species. The most logical means of accommodating all known herpesviruses taxonomically would be to establish three families under the umbrella of a new order (*Herpesvirales*), containing herpesviruses of mammals, birds and reptiles, of amphibians and fish, and of bivalves, respectively. Since these taxa are presently a proposal and lack any formal standing, the terms mammalian, fish and bivalve herpesvirus groups are used to denote the proposed families in the following discussion.

Only three genes have clear counterparts in all three groups that are detectable by amino acid sequence comparisons. The proteins encoded by two (DNA polymerase and dUTPase) have ubiquitous cellular relatives and could have been captured independently from the host repertoire. The third gene apparently lacks a counterpart in the host cell but has distant relatives in T4 and similar bacteriophages (Davison, 1992; Mitchell *et al.*, 2002). The T4 gene is known to encode the ATPase subunit of a DNA packaging enzyme complex called the terminase (Rao and Black, 1988; Bhattacharyya and Rao, 1993), and the HSV-1 gene has properties that are consistent with a similar function (Yu and Weller, 1998).

The existence of groups of viruses that exhibit close morphological similarities but generally lack detectable genetic relationships is not unique to the herpesviruses, and may be explained as the result either of convergence

from distinct evolutionary sources or as divergence from an ancestor so ancient that sequence similarities have been obliterated. The latter hypothesis is currently favored, but the existence of a common ancestor of all herpesviruses and any contingent dates for divergence of the groups must be viewed cautiously. More speculatively, apparent similarities in aspects of DNA packaging (Booy *et al.*, 1991) and capsid maturation (Newcomb *et al.*, 1996) could be interpreted as supporting an even earlier common evolutionary origin between herpesviruses and certain double-stranded DNA bacteriophages, including T4.

Phylogenetic analyses strongly support the view that herpesviruses have largely co-evolved with their hosts, often co-speciating with them. As would be expected of evolutionary phenomena, a number of problematic observations and exceptions have emerged as data have multiplied, especially in relation to early divergences. From comparisons between the phylogenies of the viruses and their hosts, McGeoch and Cook (1994) proposed an evolutionary timescale for the *Alphaherpesvirinae* in which the *Simplex-* and *Varicellovirus* genera diverged about 73 million years ago, roughly coincident with the period of the mammalian radiation. Even at this stage, potential exceptions to the co-evolution model were apparent. For example, the taxonomical position of avian herpesviruses among the *Alphaherpesvirinae* did not fit well, and prompted the suggestion of ancient interspecies transfers between mammals and birds. In this scheme, a similar argument may be necessary to explain the position of reptilian (turtle) herpesviruses in the same subfamily (Quackenbush *et al.*, 1998; Yu *et al.*, 2001; Coberley *et al.*, 2002), especially given their distance from amphibian herpesviruses (Davison *et al.*, 1999 and unpublished data). Assuming the constancy of the molecular clock derived for the *Alphaherpesvirinae*, McGeoch *et al.* (1995) tentatively dated the divergence of the *Alpha-*, *Beta-* and *Gammaherpesvirinae* at 180–220 million years ago. Given the contrasting lack of relationships between the groups and substantial relationships within them (see below), this date did not fit well qualitatively with a model in which the fish and mammalian herpesvirus groups co-speciated when teleosts separated from other vertebrates. In a recent analysis utilizing improved algorithms and the latest estimates for host divergence dates, McGeoch and Gatherer (2005) pushed back the common ancestor of the *Alpha-*, *Beta-* and *Gammaherpesvirinae* to about 400 million years ago, which permitted a greater degree of support for co-evolution of the *Alphaherpesvirinae*, including avian and reptilian members. In this scheme, a much earlier, non-co-speciative divergence may be indicated for the mammalian and fish herpesvirus groups (along with one of similar or greater antiquity for the bivalve herpesvirus group). However, this would lack the advantage of explaining the segregation of the viral groups to distinct parts of the animal kingdom and necessitate additional arguments involving viral extinction.

The mammalian herpesvirus group

In contrast to the lack of extensive relationships between the three groups, members of the mammalian herpesvirus group are clearly related to each other (Davison, 2002), as are those in the fish herpesvirus group (Bernard and Mercier, 1993; Davison, 1998; Davison *et al.*, 1999; Waltzek *et al.*, 2005, and unpublished data). Figure 2.2 shows the gene layout in representatives of two genera for each of the three subfamilies in the mammalian herpesvirus group. The subfamilies share 43 genes, termed "core genes," which were presumably inherited from a common ancestor (McGeoch and Davison, 1999). This number assumes a small degree of approximation, since amino acid sequence conservation among the set varies from substantial to marginal. The core genes are shaded grey in Fig. 2.2, and are largely confined to the central regions of the genomes, as is especially apparent with HCMV. Accumulation of more recently evolved genes near the termini is a feature of linear, double-stranded DNA genomes from other virus families, such as the *Poxviridae* (Upton *et al.*, 2003; McLysaght *et al.*, 2003; Gubser *et al.*, 2004) and the *Adenoviridae* (Davison *et al.*, 2003c), and also of eukaryotic chromosomes (Kellis *et al.*, 2003). The core genes are ordered similarly in the same subfamily, except for certain members of the *Alphaherpesvirinae* in which different arrangements are apparent: PRV in the *Varicellovirus* genus (Ben-Porat *et al.*, 1983; Davison and Wilkie 1983; Dezelee *et al.*, 1996; Bras *et al.*, 1999) and ILTV in the *Iltovirus* genus (Ziemann *et al.*, 1998). However, as shown in Fig. 2.2, the core genes are arranged differently in the different subfamilies, in the form of blocks, some of which are inverted (Davison and Taylor, 1987; Gompels *et al.*, 1995; Hannenhalli *et al.*, 1995). As indicated in Table 2.2, core genes are involved in vital aspects of herpesvirus growth, and many are involved directly or indirectly in DNA replication, in packaging of replicated DNA into capsids, and in capsid formation and structure. Most of the core genes are essential for growth of virus in cell culture (Ward and Roizman, 1994; Yu *et al.*, 2003; Dunn *et al.*, 2003).

Most core genes are present in all three subfamilies of the mammalian herpesvirus group, but three (encod-

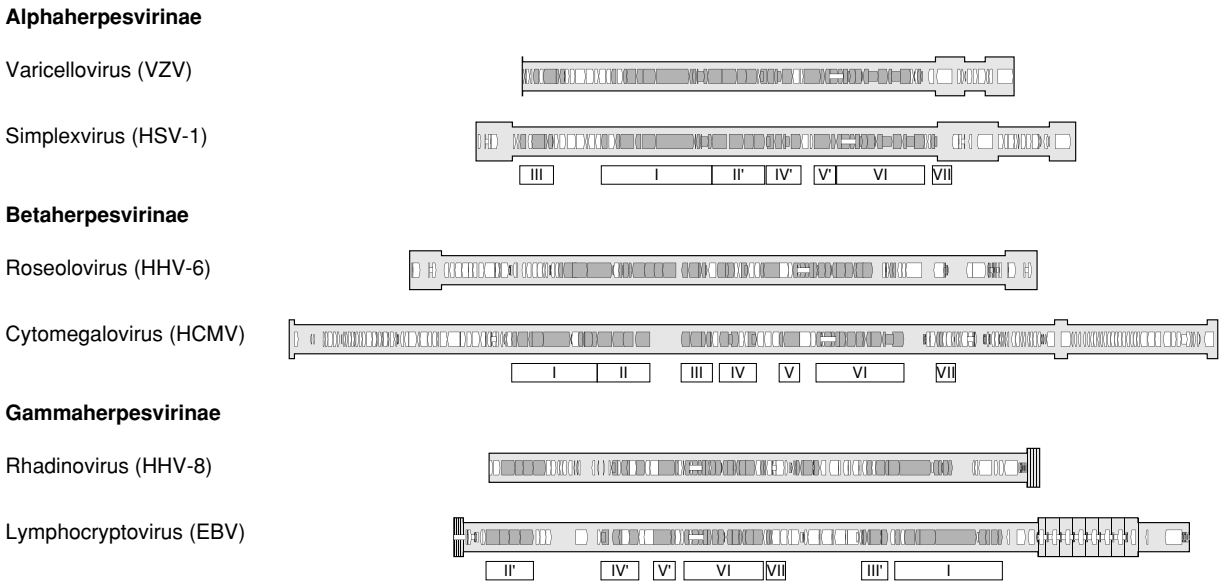

Fig. 2.2. Layout of genes in mammalian herpesvirus genomes. Repeat regions are shown in thicker format than unique regions. Protein-coding regions are shown as arrows shaded grey (core genes) or white (non-core genes), and introns as narrow white bars. Blocks of core genes that are rearranged between the subfamilies are indicated by rectangles I–VII for HSV-1, HCMV and EBV, with inverted blocks marked with a prime (Chee *et al.*, 1990). Block II also contains a local inversion and transposition of one gene (encoding DNA polymerase) that is not indicated. Genome coordinates and gene locations were obtained from accessions X04370 (VZV), X14112 (HSV-1), X83413 (HHV-6) as modified by Megaw *et al.* (1998), AY446894 (HCMV), U93872 (HHV-8) as extended at the right end of the unique region by Glenn *et al.* (1999), and AJ507799 (EBV). Variable numbers of terminal repeats are present in HHV-8 and EBV, but are shown at one end or the other other according to the accessions.

ing thymidine kinase, the small subunit of ribonucleotide reductase and the helicase that binds to the origin of DNA synthesis) have been lost from individual lineages. Thus, the origin-binding helicase gene has been retained in the *Roseolovirus* genus, but lost from other genera in the *Betaherpesvirinae*. This is mirrored in the presence of an origin of lytic DNA replication with similar structure in lineages that have retained this gene (Dewhurst *et al.*, 1993; Inoue *et al.*, 1994).

In addition to protein-coding regions, certain *cis*-acting sequences are conserved. These include the origin of lytic DNA replication, which is located similarly in each subfamily in comparison with adjacent genes, allowing for rearrangement of gene blocks. Certain members of the *Alpha-* and *Gammaherpesvirinae* contain additional lytic origins. Also, short elements near the genome termini that are involved in cleavage and packaging of unit-length genomes are conserved in all subfamilies (Broll *et al.*, 1999).

As well as the part played by the gradual processes of nucleotide substitution, insertion or deletion in generating diversity, acquisition of genes from the cell or from other viruses has played an important role throughout the evolution of the herpesviruses. There are examples of captured genes in all herpesvirus lineages. Among the mammalian herpesvirus group, the *Gammaherpesvirinae* exhibit a particularly impressive number of such genes, ranging from one encoding a product related to an enzyme involved in *de novo* purine biosynthesis (phosphoribosylformylglycineamide amidotransferase; FGARAT; Ensser *et al.*, 1997), which is present in all *Gammaherpesvirinae*, through a cyclin D gene (Nicholas *et al.*, 1992), which features in a subset of the *Rhadinovirus* genus, interferon regulatory factor genes (vIRFs), which are found only in HHV-8 and RRV (Russo *et al.*, 1996; Searles *et al.*, 1999), to a relatively recently captured core 2 β-1,6-*N*-acetylglucosaminyltransferase-mucin gene in BHV-4 (Markine-Goriaynoff *et al.*, 2003).

Duplication of genes, captured or otherwise, followed by divergence, is also apparent in all herpesvirus lineages. For example, up to three copies of the FGARAT gene are present in *Gammaherpesvirinae* (Virgin *et al.*, 1997), and HHV-8 and RRV contain four and eight vIRF genes, respectively (Searles *et al.*, 1999; Jenner *et al.*, 2001; Cunningham *et al.*, 2003). Examples of duplicated genes among other members of the mammalian herpesvirus group include

Table 2.2. Core genes in human herpesviruses, grouped according to functional class. HSV-2 and HHV-7 are not included, since their nomenclatures are the same as those for HSV-1 and HHV-6, respectively. HSV-1 and HCMV genes that are essential for growth in cell culture are marked by asterisks

HSV-1	VZV	HCMV	HHV-6	EBV	HHV-8	Function
DNA replication machinery						
UL30*	28	UL54*	U38	BALF5	9	Catalytic subunit of DNA polymerase complex
UL42*	16	UL44*	U27	BMRF1	59	Processivity subunit of DNA polymerase complex
UL9*	51	–	U73	–	–	Origin-binding protein; helicase
UL5*	55	UL105*	U77	BBLF4	44	Component of DNA helicase-primase complex; helicase
UL8*	52	UL102*	U74	BBLF2/BBLF3	40/41	Component of DNA helicase-primase complex
UL52*	6	UL70*	U43	BSLF1	56	Component of DNA helicase-primase complex; primase
UL29*	29	UL57*	U41	BALF2	6	Single-stranded DNA-binding protein
Peripheral enzymes						
UL23	36	–	–	BXLF1	21	Thymidine kinase
UL39	19	UL45[a]	U28[a]	BORF2	61	Ribonucleotide reductase; large subunit
UL40	18	–	–	BaRF1	60	Ribonucleotide reductase; small subunit
UL50	8	UL72[a]	U45[a]	BLLF3	54	Deoxyuridine triphosphatase
UL2	59	UL114	U81	BKRF3	46	Uracil-DNA glycosylase
Processing and packaging of DNA						
UL12	48	UL98*	U70	BGLF5	37	Deoxyribonuclease; role in DNA maturation and recombination
UL15*	42/45	UL89*	U66	BGRF1/BDRF1	29	Putative ATPase subunit of terminase; capsid-associated
UL28*	30	UL56*	U40	BALF3	7	Putative subunit of terminase; *pac* site-specific binding; capsid-associated
UL6*	54	UL104*	U76	BBRF1	43	Portal protein; forms dodecameric ring at capsid vertex; complexed with terminase
UL25*	34	UL77*	U50	BVRF1	19	Possibly caps the portal after DNA packaging is complete; tegument protein
UL32*	26	UL52*	U36	BFLF1	68	Involved in proper capsid localization in the nucleus
UL33*	25	UL51*	U35	BFRF1A	67A	Interacts with terminase
UL17*	43	UL93*	U64	BGLF1	32	Involved in proper capsid localization in the nucleus; tegument protein
Egress of capsids from nucleus						
UL31*	27	UL53*	U37	BFLF2	69	Nuclear matrix protein; component of capsid docking complex on nuclear lamina
UL34*	24	UL50*	U34	BFRF1	67	Inner nuclear membrane protein; component of capsid docking complex on nuclear lamina
Capsid assembly and structure						
UL19*	40	UL86*	U57	BcLF1	25	Major capsid protein; component of hexons and pentons
UL18*	41	UL85*	U56	BDLF1	26	Component of intercapsomeric triplex between hexons and pentons
UL38*	20	UL46*	U29	BORF1	62	Component of intercapsomeric triplex between hexons and pentons
UL35	23	UL48A*	U32	BFRF3	65	Small capsid protein located on tips of hexons; interacts with dynein and microtubules
UL26*	33	UL80*	U53	BVRF2	17	Maturational protease; generates mature forms of scaffolding proteins
UL26.5	33.5	UL80.5	U53.5	BdRF1	17.5	Scaffolding protein removed from capsid during DNA packaging
Tegument						
UL7	53	UL103	U75	BBRF2	42	Associated with intracellular capsids
UL11	49	UL99*	U71	BBLF1	38	Role in virion egress and secondary envelopment in the cytoplasm; myristylated and palmitylated protein; interacts with UL16 protein
UL14	46	UL95*	U67	BGLF3	34	Interacts with UL11 protein; regulates UL13 protein kinase
UL16	44	UL94*	U65	BGLF2	33	Interacts with UL11 protein; regulates UL13 protein kinase
UL21[b]	38	UL88	U59	BTRF1	23	
UL36*	22	UL48*	U31	BPLF1	64	Huge virion protein; interacts with UL37 protein; influences release of DNA from capsids during entry

Table 2.2. (*cont.*)

HSV-1	VZV	HCMV	HHV-6	EBV	HHV-8	Function
UL37*	21	UL47	U30	BOLF1	63	Interacts with UL36 protein
UL51	7	UL71*	U44	BSRF1	55	
Surface and membrane						
UL27*	31	UL55*	U39	BALF4	8	gB
UL1*	60	UL115*	U82	BKRF2	47	gL; complexed with gH
UL22*	37	UL75*	U48	BXLF2	22	gH; complexed with gL
UL10	50	UL100*	U72	BBRF3	39	gM; complexed with gN
UL49A[c]	9A	UL73*	U46	BLRF1	53	gN; complexed with gM; not glycosylated in some herpesviruses
Control and modulation						
UL13	47	UL97	U69	BGLF4	36	Serine–threonine protein kinase; tegument protein
UL54*	4	UL69	U42	BSLF1/BMLF1	57	Multifunctional regulator of gene expression
Unknown						
UL24	35	UL76*	U49	BXRF1	20	Nuclear protein

[a] Probably not an active enzyme, as catalytic residues are absent.
[b] This assignment is tentative and is excluded from the total of 43 core genes given in the text. It depends on positional, rather than sequence, conservation, and is compromised by that fact that UL21 is not flanked in each subfamily by clear homologues, unlike other core genes assigned on a positional basis.
[c] Also referred to as UL49.5.

a family of glycoprotein genes in the *Alphaherpesvirinae* (McGeoch, 1990) and 12 families, each containing up to 14 genes, in HCMV as a representative of the *Betaherpesvirinae* (Dolan *et al.*, 2004). There are four gene families in the fish herpesvirus, CCV (Davison, 1992), and 12 in the bivalve herpesvirus, OsHV-1 (Davison *et al.*, 2005). Given that gene duplication has been widely employed in host evolution (Prince and Pickett, 2002), and the greater evolutionary rates of herpesviruses, it seems likely that this means for generating diversity has played a greater part in herpesvirus evolution than can be detected by primary sequence comparisons.

The *Alphaherpesvirinae* subfamily

The employment of gene capture and duplication among the *Beta-* or *Gammaherpesvirinae* to generate diversity has received extensive attention in the literature (for details, see Chapters 15 and 22). In contrast, the *Alphaherpesvirinae* have evolved less adventurously in terms of gene content since their divergence from a common ancestor, and it is clear that gene loss has occurred. This mode of survival is considered in the following paragraphs.

The *Alphaherpesvirinae* contain four genera, plus the reptilian herpesviruses. Several complete genomes have been sequenced for the *Simplexvirus*, *Varicellovirus* and *Mardivirus* genera (Table 2.1). Data for the *Iltovirus* genus are more sparse. Limited sequence data are available for reptilian herpesviruses. Figure 2.3 illustrates the genetic content of members of the *Varicello-*, *Simplex-* and *Mardivirus* genera. Three examples (VZV, EHV-1 and BHV-1) were chosen to represent the major lineages in the *Varicellovirus* genus (see Chapter 1), plus SVV as a close relative of VZV. Core genes are shown in grey, and other genes that have counterparts in all three genera are shown in white. All of these genes were presumably present in the common ancestor, which is estimated to have existed 135 million years ago (McGeoch and Gatherer, 2005), and they comprise all or nearly all of the genes in extant *Alphaherpesvirinae*. It seems that only a few genes have developed since that era, and that the most of these are located near the genome termini.

Figure 2.4 shows a scheme of relationships between the genes at the left end of the genome, based on sequence conversation and the observation that two genes (UL56 and ORF1) encode potential membrane proteins. Also included are data for PRV (whose closest sequenced relative is BHV-1), MDV-2 and ILTV. Since U_L inverts in HSV-1, and U_L in the prototype genome orientation turned out to be inverted in comparison with the *Varicellovirus* genus (Hayward *et al.*, 1975; Wadsworth *et al.*, 1975), the genes at the left end of HSV-1 U_L are presented in the reverse order. In Fig. 2.4,

Fig. 2.3. Layout of genes in genomes of the *Alphaherpesvirinae*. Repeat regions are shown in thicker format than unique regions. Protein-coding regions are shown as arrows shaded grey (core genes), white (other genes shared by two or more genera) or black (other genus-specific genes that have presumably evolved more recently), and introns as narrow white bars. Genome coordinates and gene locations were obtained from accessions listed in the legend to Fig. 2.2, and from AF275348 (SVV), M86664 (EHV-1), AJ004801 (BHV-1) and AF243438 (MDV-1).

UL56 and UL55 thus precede UL54, which is a core gene. VZV has two extra genes (ORF1 and ORF2) sandwiched between UL55 and UL56, and the other viruses have between one and four genes in this region. For example, SVV lacks ORF2 and has a partial duplication of UL54 near the end of the genome. A parsimonious approach indicates that the ancestor preceding divergence of the *Iltovirus* genus had at least one of the genes at the left genome terminus (UL56; 180 million years ago; McGeoch and Gatherer, 2005), that the ancestor preceding divergence of the *Mardivirus* genus had at least three (UL56 (since lost), UL55 and ORF2; 135 million years ago), that the ancestor of the *Varicellovirus* and *Simplexvirus* genera had at least three (UL56, UL55 and ORF2; 120 million years ago) and that the ancestor of VZV and EHV-1 had all four genes (82 million years ago). Various of these genes have been lost during subsequent evolution of the mammalian viruses.

Gene loss is also apparent at the right genome terminus (Fig. 2.4), where, again, few genes are specific to one virus or a few closely related viruses. Even the more recently evolved genes may have substantial histories. Of the three HSV-1 genes in this category, two at the right end of U_S (US11 and US12) have counterparts in related viruses of monkeys (HVB and SA8; Ohsawa *et al.*, 2002; Tyler *et al.*, 2005). HSV-1 and HVB are considered to have co-speciated with their hosts about 23 million years ago (McGeoch *et al.*, 2000). The gene at the left genome terminus (RL1; repeated internally) has a counterpart at a similar location in a wallaby herpesvirus genome (Guliani *et al.*, 2002).

Outlook

Investigation of the genome structures, genetic contents and evolution of herpesviruses is a maturing field that undergirds the rest of herpesvirology. As with other complex analytical subjects, future advances will require incisive examination of both new data and the framework into which they are fitted. There is yet room for more surprises.

"It is a mistake to try to look too far ahead. The chain of destiny can only be grasped one link at a time." *Winston Churchill.*

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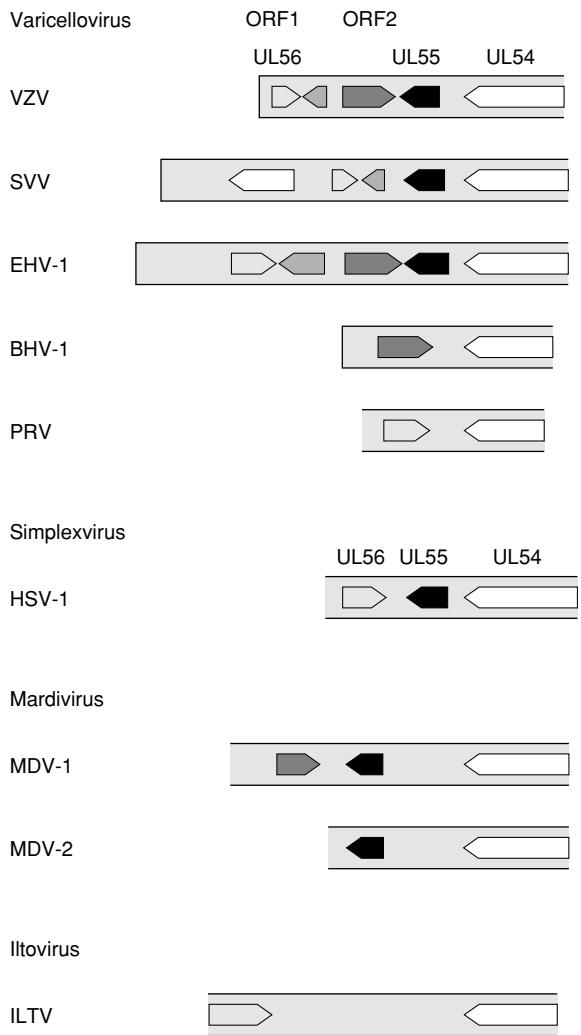

Fig. 2.4. Layout of genes at or near the left terminus in genomes of the *Alphaherpesvirinae*. The left terminus, where included, is shown by a vertical line. Homologous genes are shaded equivalently, and their nomenclature is indicated. An additional non-homologous gene is present in the *Mardi-* and *Iltovirus* genomes down stream from UL55, but is not shown. Gene locations were derived or deduced from accessions listed in the legend to Fig. 2.3, and from BK001744 (PRV), AB049735 (MDV-2) and U80762 (ILTV).

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3

Comparative virion structures of human herpesviruses

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Introduction

The herpesvirus family consists of a group of viruses distinguished by the large size of their linear double-stranded DNA genomes (~130–250 kbp) and a common architecture of infectious particles (Fig. 3.1) (Chiu and Rixon, 2002; Gibson, 1996; Steven and Spear, 1997). Indeed, before the birth of molecular biology and the availability of genomic sequencing, the common hallmark structural features shared by these viruses were the most important criteria for the classification of a herpesvirus (Roizman and Pellett, 2001). All herpesviruses identified to date, which include eight different types that are known to infect human, and more than 170 other viruses that are found in animals as well as in fish and amphibians (Roizman and Pellett, 2001), exhibit identical structural design as illustrated using human cytomegalovirus shown in Fig. 3.1. These viruses have a highly ordered icosahedral-shape nucleocapsid of about 125–130 nm in diameter, which encases the viral DNA genome. The nucleocapsid is surrounded by a partially ordered proteinaceous layer called the tegument, which in turn is enclosed within the envelope, a polymorphic lipid bilayer containing multiple copies of more than 10 different kinds of viral glycoproteins that are responsible for viral attachment and entry to host cells.

Based on their biological properties such as growth characteristics and tissue tropism, herpesviruses can be further divided into three subfamilies. Among the eight human herpesviruses, the alpha subfamily includes neurotropic viruses and contains the herpes simplex virus (HSV) 1 and 2, and Varicella zoster virus (VZV). The members of the gamma subfamily are lymphotropic viruses and include Epstein–Barr virus (EBV) and Kaposi's sarcoma-associated herpesvirus (KSHV). The viruses of the beta subfamily appear to be able to establish infections in many different types of cells and tissues, and include human cytomegalovirus (HCMV), and human herpesvirus 6 and 7. This subfamily classification system is largely consistent with the extensive genomic information that is now available (McGeoch *et al.*, 2000). While studies have been attempted to investigate the structure and architecture of each of the eight human herpesviruses, virion and virus-related particles of herpes simplex virus 1 (HSV-1), the prototype of all herpesviruses, have been subjected to the most extensive structural studies (Booy *et al.*, 1991; Newcomb *et al.*, 1993, 2000; Schrag *et al.*, 1989; Trus *et al.*, 1996; Zhou *et al.*, 1999, 2000). During the last several years, significant progress has also been made in understanding the structure of cytomegaloviruses (Bhella *et al.*, 2000; Chen *et al.*, 1999; Trus *et al.*, 1999), the prototype of the beta herpesvirus family, and KSHV, a representative of the gamma herpesvirus family (Lo *et al.*, 2003; Nealon *et al.*, 2001; Trus *et al.*, 2001; Wu *et al.*, 2000). Using HSV-1, HCMV, and KSHV as examples for each of the subfamilies, this chapter focuses primarily on the structures of these three viruses, and discusses the recent progress on understanding the structures of human herpesviruses.

Different virus-related particles found in infected cells

Summary of virion assembly pathway

Each of the herpesviruses encodes a specific set of proteins that form the different compartments of the virion (e.g. capsid, Table 3.1). Although many of the primary amino acid sequences of these proteins are not highly conserved among different viruses, the assembly pathway of the virus particles is highly similar (Fig. 3.2) (Gibson, 1996; Roizman and Knipe, 2001; Yu *et al.*, 2003). The nucleocapsid

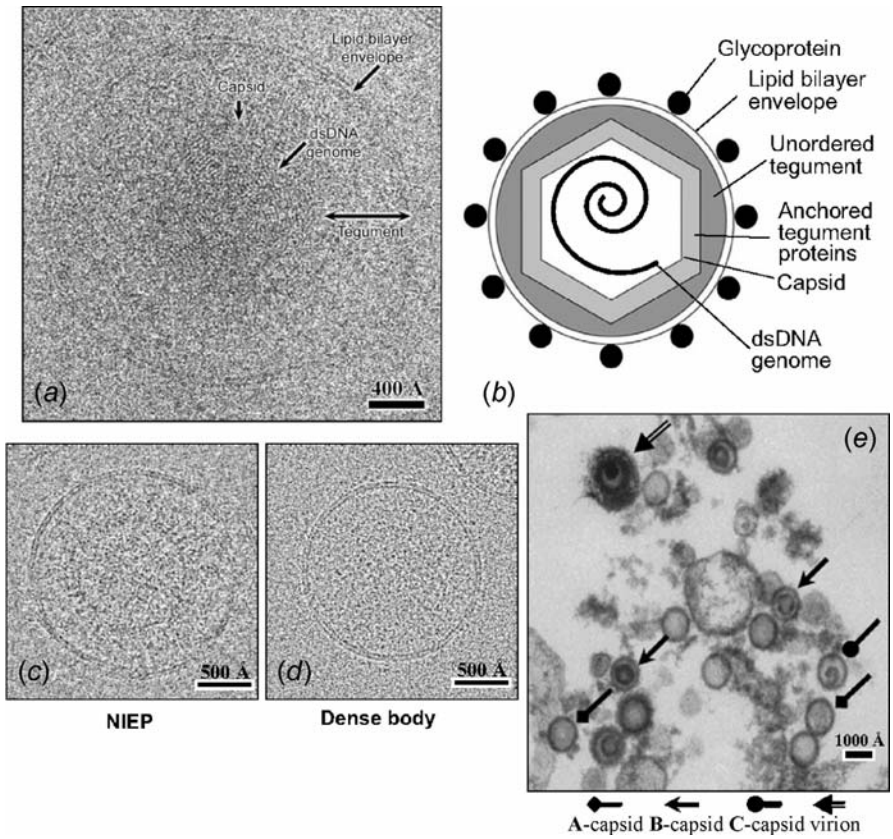

Fig. 3.1. Herpesvirus architecture. (*a*) Electron cryomicrograph of a human cytomegalovirus virion showing the different compartments of a herpes virion. (*b*) Schematic diagram illustrating the multilayer organization of human herpesviruses. Also shown are the electron cryomicrograph of a non-infectious enveloped particle (NIEP) (*c*) and a dense body (*d*) isolated from HCMV virion preparations. (*e*) Virions and different kinds of capsids observed in the thin sections of human foreskin fibroblasts infected with HCMV by negative stain electron microscopy.

is formed in the nucleus and follows a pathway that bears a marked resemblance to those of DNA bacteriophages (Casjens and Hendrix, 1988). First, a procapsid is assembled with the formation of the capsid shell and the internal scaffolding structure. Second, the procapsid is converted into mature nucleocapsid, during which time, the morphogenic internal scaffolding protein is released and replaced by the viral DNA genome, concomitant with a major conformation change of the capsid shell (Newcomb *et al.*, 1999; Yu *et al.*, 2005). Subsequent events, however, differ from the phage assembly pathway (Fig. 3.2). The mature nucleocapsid exits the nucleus and acquires its tegument and envelope, through repeated fusion with and detachment from nuclear membranes and other cellular membranous structures. Eventually, the mature infectious virion particles are released into the extracellular space via cellular secretory pathways. During this assembly process, different virus-related particles and structures, including the mature nucleocapsids and virions as well as the intermediate and aberrant products, can be found in the infected cells and the extracellular media (Figs. 3.1(*c*)–(*e*) and 3.2).

Different virus-like particles secreted from infected cells

Since the discovery of the herpesviruses, it has been long recognized that, in addition to producing infectious virus particles, the infected host cells also generate noninfectious particles such as noninfectious enveloped particles (NIEP, Fig. 3.1(*c*)) and dense bodies (DB) (Figs. 3.1(*d*) and 3.2) (Gibson, 1996; Steven and Spear, 1997). Both NIEP and DB are commonly found in the culture media of cells that are lytically infected with HSV-1 and HCMV. The ratio

Table 3.1. Major virion proteins present in HSV-1, HCMV and KSHV

		HSV-1			HCMV			KSHV			
Location	Common name	Protein name	ORF	Size (aa)	Protein name	ORF	Size (aa)	Protein name	ORF	Size (aa)	
inside the capsid	protease	protease	UL26	635	NP1c	UL80a	708	Pr	ORF17	553	~100
	Scaffolding	VP22a	UL26.5	329	AP	UL80.5	373	AP	ORF17.5	283	~1200 in B-capsid, 0 in A- & C-capsids
	MCP	VP5	UL19	1374	MCP	UL86	1370	MCP	ORF25	1376	960; penton & hexon subunit
on the Capsid shell	TRI-2	VP23	UL18	318	mCP	UL85	306	TRI-2	ORF26	305	640, dimer in triplex
	TRI-1	VP19c	UL38	465	mCBP	UL46	290	TRI-1	ORF62	331	320, monomer in triplex
	SCP	VP26	UL35	112	SCP	UL48.5	75	SCP	ORF65	170	900; hexon tip

Abbreviations: MCP, major capsid protein; TRI-2, triplex dimer protein; TRI-1, triplex monomer protein; SCP, smallest capsid protein; Pr, protease; AP, assembly protein.

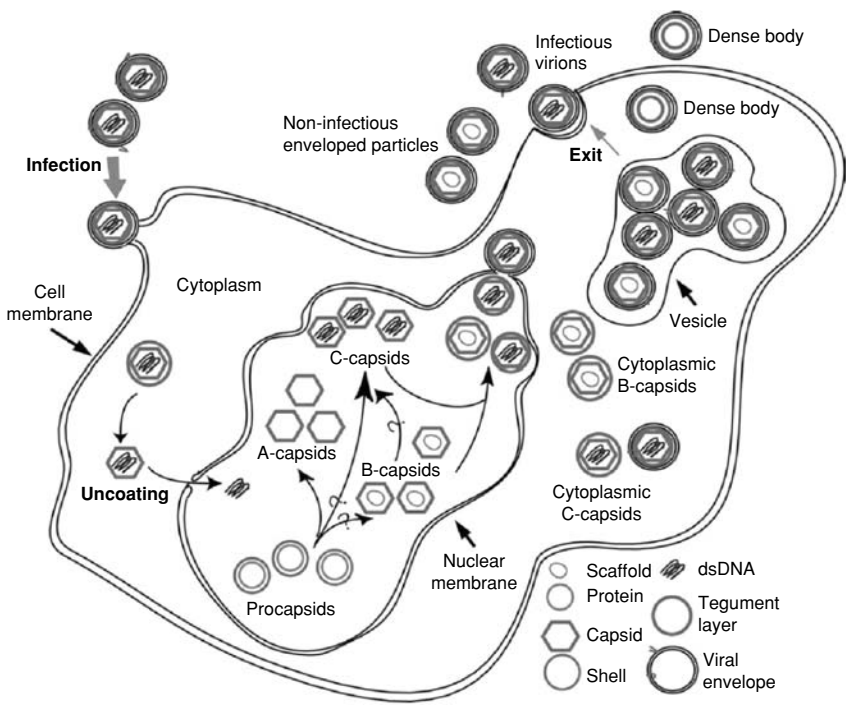

Fig. 3.2. Different virus-like particles and structures during lytic cycle of herpesvirus replication. The infectious virion initializes infection by either endocytosis or fusion with the cell membrane, which releases the nucleocapsid and some tegument proteins into the cytoplasm. The nucleocapsid is uncoated and transported across the cytoplasm (Sodeik *et al.*, 1997), allowing injection of the viral DNA through nuclear pores into the nucleus, where replication and capsid assembly take place. Procapsids mature into the C-capsid by encapsidating the viral dsDNA. Failure of DNA encapsidation results in the abortive A-capsid. Both B-capsid and C-capsid can acquire a layer of tegument proteins at the nuclear membrane of the host cell to become cytoplasmic capsids, and are enveloped and released by exocytosis to become non-infectious (NIEP) and infectious particles, respectively. Dense bodies, which contain a large amount of tegument proteins but no capsids or viral DNA, can also be found in the extracellular media.

of these particles to mature infectious virion particles can sometimes reach 20:1, suggesting that they are produced in great excess (Gibson, 1996; Steven and Spear, 1997). The exact function of these non-infectious particles in viral infection and replication is currently unknown, although they have been proposed to act as decoys that saturate and overwhelm the immune surveillance thereby facilitating the survival of the infectious virions in the hosts (Gibson, 1996; Steven and Spear, 1997).

Structurally, both the NIEP and DB are significantly different from the infectious virion (*cf.* Fig. 3.1(*a*), (*c*), (*d*)). They can be easily distinguished using electron cryomicroscopy (cryoEM) and separated from the mature infectious virions using ultracentrifugation approaches. As described above, all infectious herpesvirus virions share four common structural features (Fig. 3.1(*b*)). First, all herpesviruses contain a large double-stranded DNA (dsDNA) genome. The genomic DNA represents a dense core of ~90 nm in diameter, which can be stained with uranyl acetate and visualized using electron microscopy (Gibson, 1996; Steven and Spear, 1997) and appears as "fingerprint" patterns when examined by electron cryomicroscopy (Fig. 3.1(*a*)) (Booy *et al.*, 1991; Zhou *et al.*, 1999). Second, a capsid of icosahedral shape, which primarily consists of many copies of four different viral proteins, encases the genomic DNA. Third, a protein layer structure, named as the tegument first by Roizman and Furlong (Roizman and Furlong, 1974), surrounds the capsid and occupies the space between the capsid and the envelope. The tegument structure contains many virus-encoded factors that are important for initiating viral gene transcription and expression as well as modulating host metabolism and shutting down host antiviral defense mechanism (for a brief review, see Roizman and Sears, 1996). Finally, a lipid-bilayer envelope constitutes the outermost perimeters of the particles, and contains all the surface virion glycoproteins that are responsible for viral infectivity and entry (Fig. 3.1).

Unlike infectious virion particles, a NIEP does not contain a genomic DNA core and its capsid core appears to be B-capsid-like under electron microscopy (Figs. 3.1(*c*) and 3.2). In contrast, a dense body does not contain a capsid and appears as a cluster of tegument proteins encased by the lipid-bilayer membranous envelope (Fig. 3.1(*d*)). The presence of NIEP and DB indicates that neither packaging of viral genome nor capsid formation is required for viral envelopment.

Different capsid-like structures inside the infected cells

The capsid assembly is a continuous sequential process, leading to the synthesis of the highly ordered capsid structures. In cells that are lytically infected with herpesviruses, several kinds of virus capsid-like structures have been identified as representing stable endpoints or long-lived states (Figs. 3.1 and 3.2). Gibson and Roizman first introduced the terms A-, B-, and C-capsids to describe these intracellular capsid-like structures in HSV-1 infected cells (Gibson and Roizman, 1972). Similar capsid structures have been observed in cells infected with HCMV (Gibson, 1996; Irmiere and Gibson, 1985). Recent work has revealed A-, B- and C-capsids of comparable chemical composition and structural features in the nuclei of gammaherpesvirus infected cells and this suggests that the gammaherpesvirus capsid assembly probably also proceeds in a similar manner (O'Connor *et al.*, 2003; Yu *et al.*, 2003). These capsids all have a distinctive polyhedral shape when examined under electron microscope. Another capsid type, termed procapsid, can be obtained from in vitro assembly experiments using recombinant capsid proteins or from cells infected by a HSV-1 mutant containing a temperature sensitive mutation at the gene encoding the viral protease (Rixon and McNab, 1999; Trus *et al.*, 1996). The procapsid has a distinctive spherical shape and is only transiently stable. They undergo spontaneous structural rearrangement to become the stable angular or polyhedral form similar to the other types of capsids (Heymann *et al.*, 2003; Yu *et al.*, 2005; Zhou *et al.*, 1998b). A-capsids represent empty capsid shells that contain neither viral DNA nor any other discernible internal structure. They are thought to arise from abortive, dead-end products derived from either the inappropriate loss of viral DNA from a C-capsid or the premature release of scaffolding protein from a B-capsid without concurrent DNA packaging (Gibson, 1996). B-capsids are capsid shells containing an inner array of scaffolding protein. C-capsids are mature capsid shells that are packaged with viral DNA and do not contain the scaffolding proteins. B-capsids are believed to be derived from the procapsids upon proteolytic cleavage of the scaffolding protein, and their fate in viral maturation is controversial. Early pulse-chase experiments have suggested that B-capsids can mature to C-capsids, which in turn serve as the infectious virus precursors (Perdue *et al.*, 1976), and recent studies suggest that they might also be a dead-end product in capsid assembly similar to A-capsids (Trus *et al.*, 1996; Yu *et al.*, 2004). It remains unclear whether the spherical procapsids first adapt to the stable angular form before or after the cleavage of its scaffolding protein. The C-capsid buds through the nuclear membrane using an envelopment and de-envelopment process and acquires an additional layer of proteins that forms the tegument in the cytoplasm (for review, see Mettenleiter, 2002). Enveloped virions are then released by exocytosis (Fig. 3.2).

Assembly of viral capsid

A-, B- and C-capsids represent the stable intermediates or the end products of the herpesvirus capsid assembly process (Figs. 3.1(*e*) and 3.2). In HSV-1, capsid assembly begins with the formation of the spherical procapsid through the association of the carboxyl terminus of the scaffolding protein with the amino terminus of the viral major capsid protein (MCP), similar to bacteriophage proheads (Conway *et al.*, 1995; Jiang *et al.*, 2003). Previous experiments have shown that the procapsid can be assembled in vitro from the capsid and scaffolding proteins, in the absence of the viral capsid maturation protease (Newcomb *et al.*, 1999) or from cells infected with viruses containing a temperature-sensitive protease mutant (Heymann *et al.*, 2003). These procapsids can spontaneously rearrange into a large-cored, angular particle resembling the B-capsid, but these large-cored particles do not encapsidate DNA or become mature virions. Past studies have also shown that cells infected with a HSV-1 mutant containing a temperature-sensitive mutation in the protease gene produced capsids that assemble at the non-permissive temperature, similar to the in vitro-assembled procapsids (Rixon and McNab, 1999). The capsids matured when protease activity was restored (Rixon and McNab, 1999), demonstrating that the procapsid is the precursor to the angular capsid (Fig. 3.2). The proteolytic cleavage of the intra-capsid scaffolding proteins at their C-termini by the viral protease (Hong *et al.*, 1996; Liu and Roizman, 1991, 1992; Preston *et al.*, 1992; Welch *et al.*, 1991) interrupts the interactions between the scaffolding proteins and the major capsid proteins (Zhou *et al.*, 1998b). The interactions between the scaffolding protein, the major capsid protein, and viral protease are important targets for antiviral drug design in treating and controlling herpesvirus infections (Flynn *et al.*, 1997; Qiu *et al.*, 1996; Shieh *et al.*, 1996; Tong *et al.*, 1996, 1998). Proteolytic cleavage of the scaffolding protein is followed by the recruitment of the smallest capsid protein, VP26, through an ATP-dependent process (Chi and Wilson, 2000), leading to the formation of the intermediate or B-capsids. The mature procapsids are believed to arise spontaneously by packaging the viral genome DNA, a process that is currently not completely understood (Yu *et al.*, 2005).

Compositions and three-dimensional structural comparisons of alpha, beta and gammaherpesvirus capsids

A-, B-, and C-capsids (Yu *et al.*, 2005) can be isolated from the nucleus of the host cells lytically infected by herpesviruses and they have been subjected to three-dimensional structure studies for HSV-1 (Zhou *et al.*, 1998a, 1994), HCMV (Butcher *et al.*, 1998; Chen *et al.*, 1999; Trus *et al.*, 1999), and KSHV (Nealon *et al.*, 2001; Trus *et al.*, 2001; Wu *et al.*, 2000; Yu *et al.*, 2003). While these three types of capsids have different composition (e.g., viral DNA and internal scaffolding protein), they all have a common shell structure that consists of 150 hexameric (hexon) and 12 pentametric (penton) capsomers, which are connected in groups of three by the triplexes, asymmetric structures that lie on the capsid floor (Fig. 3.3). During the last few years, considerable progress of the three-dimensional structure of the capsids and the assembly of the capsomers and triplexes has been made on the studies.

The capsid, approximately 1250–1300 Å in diameter, is a $T = 16$ icosahedron with 12 pentons forming the vertices, 150 hexons forming the faces and edges, and 320 triplexes interconnecting the pentons and hexons (Rixon, 1993; Steven and Spear, 1997). One of the 20 triangular faces of the icosahedral capsid is indicated by the dotted triangle in Fig. 3.3(*a*) with three fivefold ('5'), a twofold ('2') and threefold (through triplex Tf) symmetry axes labeled. The six fivefold axes pass through the vertices, the ten threefold (3f) axes pass through the centers of the faces, and the 15 twofold (2f) axes pass through the middle of the edges. The structural components in one asymmetric unit are labeled, including 1/5 of a penton ('5'), one P (peri-pentonal) hexon, one C (center) hexons a half E (edge) hexon (Steven *et al.*, 1986), and one each of Ta, Tb, Tc, Td and Te triplex and 1/3 of Tf triplex (Fig. 3.3(*a*)) (Zhou *et al.*, 1994).

HSV-1 is the easiest to grow among all human herpesviruses and has been subjected to the most thorough structural analyses, and its capsid has been reconstructed to 8.5 Å resolution (Fig. 3.3(*a*)) (adapted from Zhou *et al.*, 2000 with permission from the publisher). The capsid shell has a total mass of about 200 MDa. The structural features of the capsid are built from four of the six capsid proteins: 960 copies of the major capsid protein (MCP), VP5; 320 copies of triplex monomer protein (TRI-1), VP19c; 640 copies of triplex dimer protein (TRI-2), VP23; and 900 copies of the smallest capsid protein (SCP), VP26. At this high resolution, details of secondary structure can be resolved that are not visible at lower resolution. Alpha-helices, for example, appear as extended, cylindrical rods of 5–7 Å diameter. The VP5 major capsid protein of HSV-1 was found to contain 24 helices. These assignments of helices to densities were corroborated by docking the cryoEM structure with X-ray crystallographic data which were subsequently obtained for the upper domain of VP5 (Fig. 3.3(*c*)) (Baker *et al.*, 2003; Bowman *et al.*, 2003). A group of seven helices is clustered near the area of the protein that forms the narrowest part of the

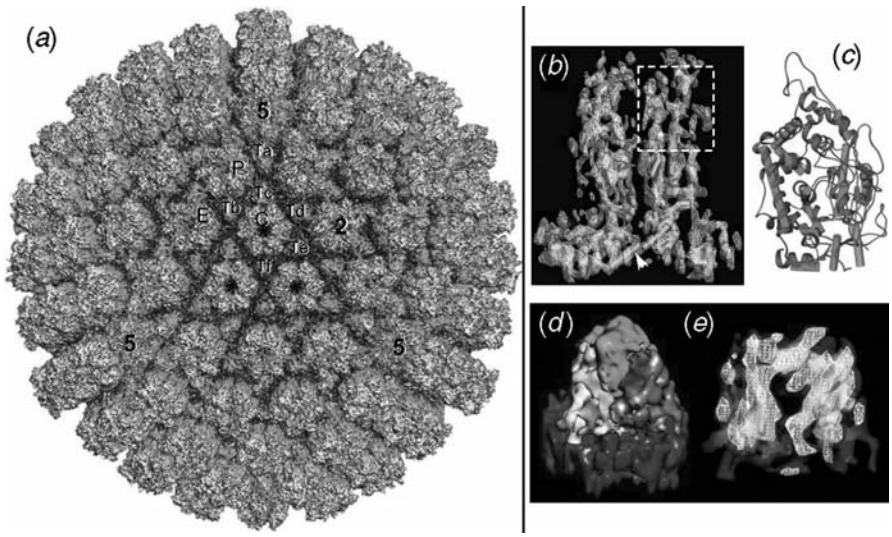

Fig. 3.3. HSV-1 capsid at 8 Å resolution (Zhou *et al.*, 2000) and atomic model of upper domain of the major capsid protein (MCP), VP5 (Bowman *et al.*, 2003). (*a*) Radially color-coded surface representation of the HSV-1 B capsid structure at 8.5 Å. One of the 20 triangular faces is denoted by dashed triangle. The penton and three types of hexons are indicated by '5', P, E and C. Also labeled are the six quasi-equivalent triplexes, Ta, Tb, Tc, Td, Te, Tf. (*b*) Two hexon subunits were shown in wire frame representation with α helices identified in one of the VP5 subunit illustrated by orange cylinders (5 Å in diameter). The red arrowhead points to the 7 helix bundle in the middle domain and the white arrow identifies the long helix in the floor domain that connects adjacent subunits.
(*c*) Ribbon representation of the atomic structure of the HSV-1 MCP upper domain determined by X-ray crystallography (Bowman *et al.*, 2003). The helices identified in the hexon VP5 subunit in the 8.5 Å HSV1 capsid map (Zhou *et al.*, 2000) are shown as cylinders: those in green match with helices present in the X-ray structure and those in yellow are absent in the X-ray model, suggesting possible structural differences of MCP packed in the crystal and inside the virion. (*d*) One single triplex is shown as shaded surface representation with individual subunits in different colors: VP19c in green and the two quasi-equivalent VP23 subunits in light and dark grey, all situated on the capsid shell domains of VP5 (blue). (*e*) α-helices identified in the two quasi-equivalent VP23 molecules (in red and yellow cylinders of 5 Å diameter, respectively). Adapted with permissions from publishers. (See color plate section.)

axial channel of the pentons and hexons (indicated by the red arrowhead in Fig. 3.3(*b*)). Shifts in these helices might be responsible for the constriction that closes off the channel to prevent release of packaged DNA. The floor domain of VP5 also contains several helices, including an unusually long one that interacts with the scaffolding core and may also interact with adjacent subunits to stabilize the capsid (arrow in Fig. 3.3(*b*)). Structural studies of in vitro assembled capsids that are representatives of capsid maturation stages suggest that substantial structural rearrangement at this region is directly related to the reinforcement of penton and hexons during morphogenesis (Heymann *et al.*, 2003).

The higher resolution of this reconstruction also revealed the quaternary structure of the triplexes, which are composed of two molecules of VP23 and one molecule of VP19c (Fig. 3.3(*d*), (*e*)). The lower portion of the triplex, which interacts with the floor of the pentons and hexons, are threefold symmetric with all three subunits roughly equivalent. This arrangement alters through the middle of the triplex such that the upper portion is composed mostly of VP23 in a dimeric configuration. It appears that all three subunits of the triplex are required for the correct tertiary structure to form because VP23 in isolation exists only as a molten globule with no distinct tertiary structure (Kirkitadze *et al.*, 1998).

The capsids of other human herpesviruses have also been studied by electron cryomicroscopy, including HCMV and simian cytomegalovirus (SCMV), and KSHV, members of the beta and gammaherpesviruses, respectively (Fig. 3.4) (Bhella *et al.*, 2000; Chen *et al.*, 1999; Trus *et al.*, 1999, 2001; Wu *et al.*, 2000). The HCMV capsid structure is very similar to HSV-1 in overall organization, with four homologous structural proteins at the same stoichiometries (Fig. 3.4(*a*) and (*b*)). The main difference is that the HCMV capsid had a larger diameter (650 Å) than HSV-1 (620 Å), resulting in a volume ratio of 1.17 (Bhella *et al.*, 2000; Chen *et al.*, 1999; Trus *et al.*, 1999). The increased size of the HCMV

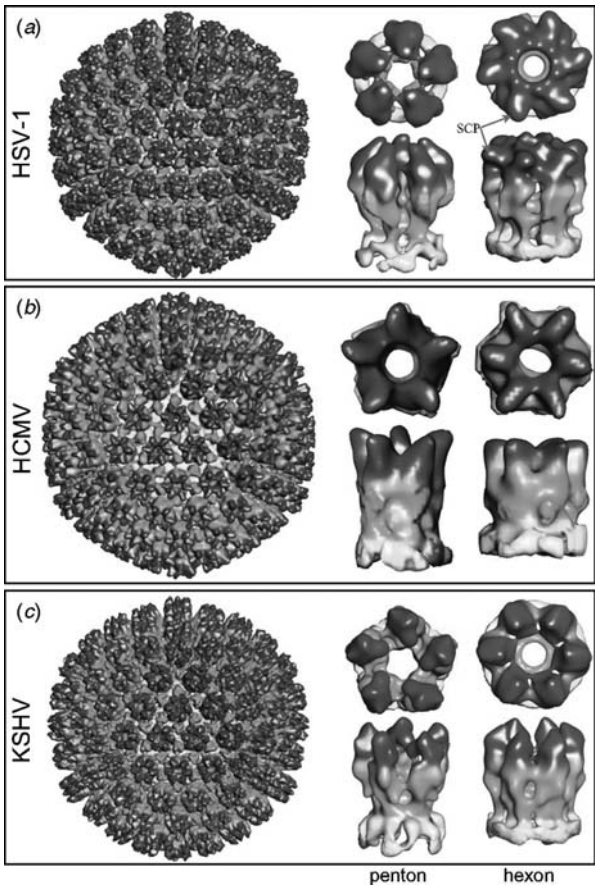

Fig. 3.4. Comparison of the three-dimensional structures of alpha, beta and gammaherpesvirus capsids. The capsid maps of HSV-1 (*a*), HCMV (*b*) and KSHV (*c*) are shown as shaded surfaces colored according to particle radius and viewed along an icosahedral three-fold axis. The resolution of the HSV-1 and KSHV capsid maps is 24 Å and that of the HCMV capsid (Butcher *et al.*, 1998) is 35 Å. The right two columns are detailed comparisons of a penton and an E hexon, which were extracted computationally from each map and shown in their top and side views. (See color plate section.)

capsid despite the similar molecular mass of its component proteins results in a greater center-to-center spacing of the capsomers compared to HSV-1 (Fig. 3.4(*b*)).

The structure of KSHV capsids was also determined by cryoEM to 24 Å resolution and exhibit structural features very similar to those of HSV-1 and HCMV capsids (Fig. 3.4(*c*)) (Trus *et al.*, 2001; Wu *et al.*, 2000). The KSHV and HSV-1 capsids are identical in size and capsomer organization. However, some notable differences are seen upon closer inspection. The KSHV capsid appears slightly more spherical than the HSV-1 capsid, which exhibits a somewhat angular, polyhedral shape. When viewed from the top, the hexons in the KSHV capsid appear flower-shaped, whereas those of HSV-1 have slightly tilted subunits and as a result appear more gear-shaped (see below). Also, the KSHV triplexes are slightly smaller and deviate less from threefold symmetry than the much-elongated triplexes in the HSV-1 capsid. The differences in the upper domains of HSV-1 and KSHV triplexes indicate that the HSV-1 triplexes are slightly taller. The radial density profiles show that the KSHV and HSV-1 capsids have identical inner radii of 460 Å (Wu *et al.*, 2000). Because both viruses also have similar genome sizes, their identical inner radii suggest that their DNA packing densities inside the capsids are similar. In contrast, betaherpesvirus capsids, such as those of HCMV, have a somewhat larger internal volume than HSV-1 or KSHV capsids (Bhella *et al.*, 2000; Chen *et al.*, 1999; Trus *et al.*, 1999). However, the increase in volume is disproportionate to the large increase in the size of the HCMV genome over the HSV-1 and KSHV genomes. This implies that the viral DNA is more densely packed into HCMV virions than into HSV-1 or KSHV virions.

In herpesvirus capsids, both the penton and hexon have a cylindrical shape (about 140-Å diameter, 160-Å height) with a central, axial channel approximately 25 Å in diameter (Fig. 3.4). The penton and hexon subunits both have an elongated shape with multiple domains, including upper, middle, lower, and floor domains. The middle domains of the subunits interact with the triplexes. The lower domains connect the subunits to each other and form the axial channels. While the upper domains of adjacent hexon subunits interact with one another, adjacent penton subunits are disconnected at their upper domains, resulting in the V-shaped side view of the pentons (Fig. 3.4). Another major difference between the penton and hexon concerns their floor domains. These domains play an essential role in maintaining capsid stability, as suggested by the higher-resolution structural studies of the HSV-1 capsid (Zhou *et al.*, 2000), where a long α-helix inserts into the floor domain of the adjacent subunit (Fig. 3.3(*b*)). The relative angle between the floor and lower domains is about 110° in the penton subunit and becomes less than 90° in the hexon subunit, making the penton to appear longer in its side view.

The HSV-1 penton and hexon subunits have the same basic shape as the HCMV and KSHV subunits (Fig. 3.4). Each consists of upper, middle, lower, and floor domains. However, the upper domains of the HSV-1 penton subunits point inward toward the channel, whereas those of the HCMV and KSHV penton subunits point outward. The upper domain of the KSHV subunit has a rectangular

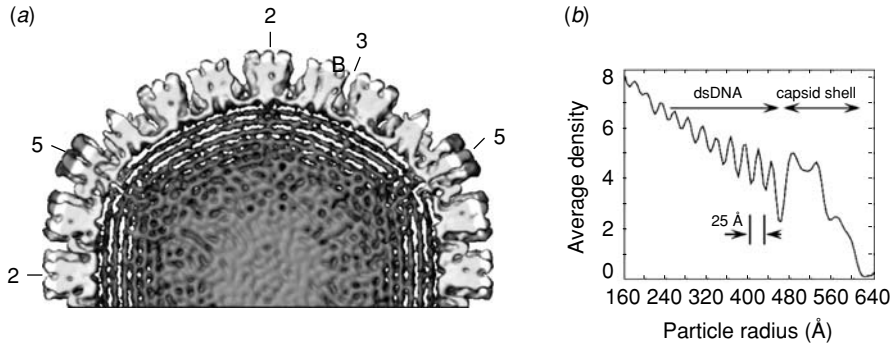

Fig. 3.5. Packing of dsDNA inside herpesvirus capsid (Yu *et al.*, 2003). (*a*) The upper half of a 100-Å thick central slice extracted from the 21 Å resolution reconstruction of the C-capsid of the rhesus rhadinovirus (RRV), a gammaherpesvirus and the closest KSHV homologue. The slice exhibits high-density features organized as multiple spherical shells inside the inner surface of the capsid floor. At least six concentric shells can be distinguished before the pattern becomes indistinct toward the center of the capsid. (*b*) Radial density distribution of the C-capsid obtained by spherically averaging the C-capsid reconstruction and plotted as a function of radius. It is evident that the distance between neighboring peaks is about 25 Å.

shape, while that of the HSV-1 penton subunit appears as a triangle. The most striking difference is that the HSV-1 hexon subunits contain an extra horn-shaped density which is not found in the HSV-1 penton (Fig. 3.4(*a*), arrow in right panel). This extra density binds to the top of each HSV-1 hexon subunit and has been shown to be the SCP, VP26, by difference imaging (Trus *et al.*, 1995; Zhou *et al.*, 1995), which associate with one another to form a hexameric ring around the hexon at a radius of approximately 600 Å. This accounts for the tilted or gear-like appearance of the HSV-1 hexon top view. The KSHV homolog of HSV-1 VP26 is ORF65. Difference map of anti-ORF65 antibody labeled and unlabeled KSHV capsids also showed that ORF65 binds only to the upper domain of the major capsid proteins in hexons but not to those in pentons (Lo *et al.*, 2003). The lack of horn-shaped densities on the hexons indicates that KSHV SCP exhibits substantially different structural features from HSV-1 SCP. The location of SCP at the outermost regions of the capsid suggests a possible role in mediating capsid interactions with the tegument and cytoskeleton proteins during infection.

Structure and packaging of viral genomic DNA

The sizes of the dsDNA genomes of different human herpesviruses vary substantially, e.g., the HCMV genome is 51% longer than HSV-1 (Davison *et al.*, 2003; McGeoch *et al.*, 2000). The major point of interest concerns the packing of their genomes within the capsids. The HCMV capsid is 117% larger than HSV-1. Besides the volume, factors such as DNA density, capsid capacity, and capsid expansion can also influence DNA packaging in viruses. The genome of HCMV might be more densely packed than that of HSV-1, or might induce expansion of the capsid upon packaging. Alternatively, the two viruses might have a similar capacity but differ in the amount of unoccupied space at the center of the capsids.

In HSV-1, the genomic DNA within the nucleocapsid is closely packed into multiple shells of regularly spaced densities, with 26 Å between adjacent DNA duplexes (Zhou *et al.*, 1999). The central slice and radial density plot in Fig. 3.5 indicate that the C-capsid of Rhesus rhadinovirus (RRV), a gammaherpesvirus, has an almost identical pattern of DNA organization to those observed in HSV-1, though slightly more compact, with a 25-Å inter-duplex distance (Yu *et al.*, 2003). Although the RRV capsid, like the KSHV capsid, has nearly the same diameter as the HSV-1 capsid (1250 Å), RRV has a slightly larger genome size than HSV-1, ~165 vs. 153 kb, respectively (Alexander *et al.*, 2000; Lagunoff and Ganem, 1997; Renne *et al.*, 1996; Searles *et al.*, 1999). Therefore, the smaller inter-duplex distance may merely reflect the need to compact this greater amount of DNA into the same volume within the capsid. HCMV has the largest genome (~230 kb) of all human herpesviruses but has a capsid that is only slightly larger (1300 Å diameter), and its DNA was shown to pack with an interduplex distance of only 23 Å (Bhella *et al.*, 2000). Based on the interduplex spacing and the genome sizes, we estimate that the closely packed DNA genomes of HSV-1, RRV, and HCMV would occupy a total volume of 3.52×10^8 Å^3, 3.51×10^8Å^3, and 4.05×10^8Å^3, respectively (Yu *et al.*, 2003). These volumes would measure approximately 92%, 92%, and 90% of the total available spaces inside the HSV-1, RRV, and