HHS Public Access

Author manuscript

Curr Opin Virol. Author manuscript; available in PMC 2016 February 15.

Published in final edited form as:

Curr Opin Virol. 2014 December ; 9: 170–177. doi:10.1016/j.coviro.2014.10.004.

Roseolovirus Molecular Biology: Recent Advances

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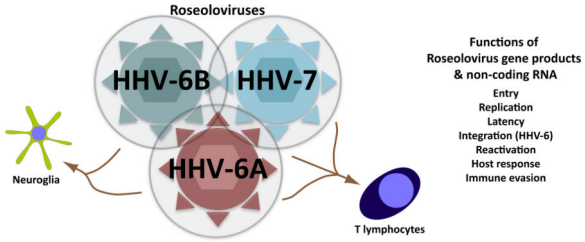
Abstract

Human herpesviruses 6A, −6B, and −7 (HHV-6A, −6B, and −7) are classified within the roseolovirus genus of the betaherpesvrus subfamily. Most humans likely harbor at least two of these large DNA viruses, and 1% of humans harbor germline chromosomally integrated HHV-6A or HHV-6B genomes. Differences at the genetic level manifest as distinct biologic properties during infection and disease. We provide a brief synopsis of roseolovirus replication and highlight the unique properties of their lifecycle and what is known about the viral gene products that mediate these functions. In the nearly 30 years since their discovery, we have only begun to unlock the molecular strategies these highly evolved pathogens employ to establish and maintain chronic infections in humans.

Graphical Abstract



The aims of this review are to provide an overview of roseolovirus molecular biology and highlight recent advances in our understanding of the molecular basis of the virus lifecycle, which in turn inform our understanding of pathogenesis, and illuminate paths to diagnosis, treatment, and prevention.

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ROSEOLOVIRUSES: WHAT ARE THEY?

Human herpesviruses 6A, 6B, and 7 (HHV-6A, HHV-6B, and HHV-7) are the only formally recognized members of genus *Roseolovirinae* within order *Herpesvirales*, family *Herpesviridae*, and subfamily *Betaherpesvirales* (Fig. 1) (historical references are available in [1,2]). HHV-6A and HHV-6B were formerly described as variants, but are now formally classified as distinct virus species by the International Committee on Virus Taxonomy [3]. Roseoloviruses share numerous genetic and biologic properties with human cytomegalovirus (also a betaherpesvirus), yet have distinct genes and disease associations (Tables 1 and 2). The human roseoloviruses are contemporary representatives of an ancient lineage of herpesviruses that co-speciated with their hosts. Antibodies against HHV-6 have been detected in several species of Old and New World monkeys, suggesting the presence of viruses related to HHV-6 in these animals [4]. Consistent with this, relatives of HHV-6 and HHV-7 have been detected by PCR in chimpanzees, other great apes, and pig-tailed macaques [5-7]

ROSEOLOVIRUSES AND HUMAN HEALTH

HHV-6B is the most common cause of roseola infantum (exanthem subitum) and related febrile rash illnesses that often accompany primary infection in early childhood [8]; this can also be caused by HHV-7. HHV-6B and HHV-7 have also been associated with febrile seizures in young children. Immune suppressed hemopoietic stem cell transplant recipients can experience limbic encephalitis and other mental disorders during HHV-6B reactivations [9]. HHV-6A has been associated with Hashimoto's thyroiditis [10] and neurological disorders, including multiple sclerosis, but proof of causality is incomplete [11].

A striking feature of roseoloviruses is the presence of mammalian telomeric sequences at the ends of the virus genome [12-14]. Approximately 1% of the human population world-wide harbors inherited chromosomally integrated (ci) HHV-6A and HHV-6B. Germline integration may be a byproduct of the use of integration as a hypothesized mechanism for establishing latency in somatic cells, with virus infection of spermatocytes leading to occasional germline transmission. The health effects of ciHHV-6 have not been elucidated.

ROSEOLOVIRUS GENOMES AND GENES

Roseoloviruses genomes consist of a long unique region (U) bracketed by a pair of direct repeats (DR) (Fig. 2). Roseolovirus genomes have heterogeneous and perfect arrays of mammalian telomeric repeats at the left and right ends of the DR elements, respectively, and consequently at the left and right genomic termini. At least for HHV-6B, genomes of wild viruses can be several kb longer than those of laboratory-adapted strains, due to repetitive sequences in the DR that are lost upon passage in cultured cells. Roseolovirus genomes are approximately 65 to 90 kb shorter than the 235 kb HCMV genome. The origins of lytic genome replication (oriLyt) are located between U41 and U42, and are structurally similar to oriLyts of alphaherpesviruses.

HHV-6A and HHV-6B are ~90% identical across their genomes, with ~95% identity across the herpesvirus core genes. Regions in the vicinity of the genomic termini are less

conserved, with as little as 50% identity in the region that encodes the major immediate early transactivators [15]. While its overall organization and gene content are similar to those of HHV-6A and HHV-6B, the HHV-7 genome is shorter and more compactly arranged across its length, with many genes being 5 to 10% shorter than their HHV-6 counterparts. In intrastrain comparisons, roseolovirus genomes are typically ~99.9% identical, except for pockets of elevated heterogeneity.

The core herpesvirus genes (43 genes conserved among members of the *Herpesviridae*) are clustered across the central portion of the genomes in an arrangement colinear with the core genes in HCMV and other betaherpesviruses. In contrast to HCMV and most other betaherpesviruses, the roseoloviruses, along with elephant endotheliotropic herpesviruses, encode homologs (roseolovirus gene U73) of the origin of DNA replication binding protein (OBP) encoded by all alphaherpesviruses. Most of the genes shared only among betaherpesviruses or unique to one or more roseoloviruses lie in or near the DR, or between conserved gene blocks (Table 2 and Fig. 2).

HHV-6B expresses several small RNAs of unknown function, including some that map to oriLyt and microRNAs that map to the DR3/B1 and B2 immediate early gene locus in DR. These miRNAs are conserved in HHV-6A, and one is an ortholog of human miRNA miR-582-5p [16].

Major functions of many roseolovirus genes are known only by inference from known functions of their homologs in HCMV or other herpesviruses. Most virion proteins are likely to have significant biological roles that go beyond structural, such as tegument proteins that modify host cell activities before *de novo* viral gene expression begins. Only a handful of genes unique to roseoloviruses have been studied functionally. These include transactivators encoded by DR6 and U3, the U94 parvovirus rep gene homolog, immunoevasins encoded by U21, a non-essential Golgi-localizing non-structural glycoprotein encoded by U23 [17], and the gQ1 and gQ2 glycoproteins.

Major research priorities include assessment of genome sequences and genetic variation of wild viruses, and identification of the functions of genes unique to roseoloviruses. A bacterial artificial chromosome (BAC) system has enabled targeted genetic analysis for HHV-6A [18]; analogous systems are needed for HHV-6B and HHV-7.

PRODUCTIVE REPLICATION

Roseolovirus tropism: beyond T cells

The human roseoloviruses were discovered on the basis of their lytic replication activity in cultured PBMCs. Some strains have adapted to growth in specific T cell lines and are commonly used for laboratory studies. Other cell types such as monocytes, dendritic cells, astrocytes, and glial cells are permissive for infection. HHV6A and HHV-6B can bind to the sperm acrosome, providing a possible route to germline integration [19]. The ability of HHV-6A and HHV-6B to infect olfactory-ensheathing glial cells that are present in the nasal cavity may provide a route to the central nervous system [20].

Mechanisms of attachment and entry are important determinants of cell tropism and latency reservoirs in the host. Each roseolovirus has a distinct entry receptor: CD46 for HHV-6A [21], CD134 for HHV-6B [22], and CD4 for HHV-7 [23]. Receptors are targets for neutralization [24] and can be used to create receptor-transgenic animal models that support infection [25]. The essential components for membrane fusion by HHV-6A and HHV-6B are gB and the gH/gL/gQ1/gQ2 complex [26-28]. gQ2 and gM are essential for virus production of HHV-6A since virus stocks could not be generated from BACs with disruptions in these ORFs [28,29]. The degree of functional homology between roseolovirus genes can be examined in transcomplementation assays and by gene substitutions in the HHV-6A BAC. For instance, the HHV-6B gH gene can functionally replace HHV-6A gH for replication [30].

De novo gene expression and productive replication

Roseolovirus lytic gene expression follows the general herpesvirus paradigm: immediate early genes are transcribed in the absence of new protein synthesis, expression of early genes is dependent on prior synthesis or immediate early proteins, and late genes are expressed at high levels upon viral DNA replication. Approximately 10 genes have spliced transcripts (some have multiple spliced isoforms), and some transcripts are kinetically regulated. Roseolovirus major IE genes are spliced and have promoters that can be highly active in T cells.

Roseoloviruses diverge from most betaherpesviruses in their mechanism of initiating viral DNA replication. Their homologs of the alphaherpesvirus origin binding protein bind to, and presumably facilitate unwinding of the origin of lytic replication to initiate viral DNA synthesis [31]. The OBPs of HHV-6B and HHV-7 have slight differences in preferential binding sites that may explain a lack of complete reciprocity between HHV-6B and HHV-7 in transient oriLyt replication assays.

Information about HHV-6 virion assembly and egress is sparse. An interesting feature of HHV-6A virion envelopes is the presence of ganglioside GM1, a component of lipid rafts [32]. Along with other evidence, this suggests that virions may assemble via lipid rafts. Envelopment and egress are via a cellular CD63-associated exosomal pathway [33].

LATENCY and REACTIVATION

Gene expression during latency

Roseolovirus latency is poorly defined in molecular terms. CD34-positive hematopoietic cells are a site of HHV-6 latency, and circulating lymphocytes positive for HHV-7 DNA but not for lytic gene transcripts have been detected. Latency associated transcripts have been identified in two loci: antisense to the major IE locus, with splicing patterns reminiscent of an HCMV latency transcript [34], and from the U94 gene [35]. No laboratory has reported detection of both of these transcripts.

Integration

One of the most unique and biologically intriguing aspects of HHV-6A and HHV-6B is their integration into the germline of some humans (~1%), which can result in inherited transmission among families [14]. All three human roseoloviruses contain mammalian telomeric sequences at their genomic termini, and telomeres are the site of integration of HHV-6A and HHV-6B in patients with chromosomally integrated HHV-6 [36,37]. Telomeric integration occurs in infected cultured Jjhan and HEK-293 cells, establishing a system for mapping and characterizing the mechanistic processes of integration. The efficiency of integration in cultured cells has led to the hypothesis that chromosomal integration is a normal part of HHV-6 latency.

The U94 gene of HHV-6A and HHV-6B is a homolog of the parvovirus Rep gene, an integrase with single-stranded and double-stranded DNA binding properties. Cytomegaloviruses of rats [38] and bats [39] encode U94 homologs, indicating that the gene may have been acquired prior to the divergence of roseoloviruses and cytomegaloviruses. HHV-6 U94 binds ssDNA [40] and its ectopic expression inhibits betaherpesvirus replication [41] and impairs lymphatic endothelial cell angiogenesis [42]. Given its homology with the parvovirus integrase, U94 is hypothesized to promote integration and excision of HHV-6A and HHV-6B, either by host-mediated base excision repair or by exonuclease strand invasion [14]. The transcriptome of ciHHV-6 cells has not been reported, but spliced U90 transcripts have been detected in B cells harboring integrated HHV-6 [43]. Genome-wide analyses of viral and cellular gene expression are needed in individuals with ciHHV-6 and in ciHHV-6 cell culture systems.

Reactivation

Uncontrolled or aberrant primary infection and HHV-6 reactivation are associated with neurological syndromes and transplant failure. Very little is known about the molecular basis of reactivation. Mitogen stimulation of PBMCs leads to reactivation and enables infection of T cell lines. Lytic replication can also be stimulated by apoptosis [44]. If integration is a mechanism of latency, a functional virus genome must be excised from telomeres in order to reactivate full lytic infection. HEK293 cells with integrated HHV-6A can produce viral genome concatamers upon treatment with the histone deacetylase inhibitor trichostatin A [36]. Huang et al. [43] noted that the telomeres attached to integrated HHV-6 genomes are frequently shortened and associated with detection of circular viral genomes. Such short, unstable telomeres are thought to facilitate excision of viral genomes via telomere-loops within the viral genome [43]. Interestingly, *Chlamydia trachomatis* drives reactivation of ciHHV-6 and transient shortening of telomere ends [45]; the signaling pathways and mechanism of excision remain to be defined.

VIRUS-HOST INTERACTIONS

All herpesviruses manipulate host cell processes to promote replication. Roseoloviruses push the cell cycle into G2/M, presumably to ramp up cellular processes that promote DNA replication [46]. Virally-induced degradation of Rb and activation of E2F1 further benefits HHV-6A and HHV-6B by enhancing the expression of some lytic genes [47]. Many

roseolovirus gene products inhibit both innate immune responses (U20, IE1) and adaptive immune responses (U21), and interfere with cell death (U19, U20, DR6) and T cell signaling (U21, U54) [48]. Functions should not be assumed to be conserved among all roseolovirus homologs. Virus-specific differences in gene function such as U54 modulation of IL-2 signaling, the chemotactic properties of the roseolovirus U83 chemokines, and IE1 inhibition of interferon stimulated genes have been noted [49-51]. BAC-based recombinant viruses will facilitate examination of gene function in the context of infection.

Roseoloviruses impact cytokine profiles of cultured cells [52,53]. Cytokine dysregulation also occurs in patients undergoing acute illness associated with primary infection [54-56] and reactivation [57], and in animal models of HHV-6 infection [25,58]. The viral gene products that induce these changes in host signaling are not known. Inactivated virions induce an interferon-lambda 1 (IL-29) response in dendritic cells that might skew T cell responses to infection [59]. The host immune response may play a large role in the immune pathology of reactivation-associated diseases and facilitate roseolovirus transit across the blood brain barrier [60]. In addition, HHV-6B reactivation might be triggered in response to pro-inflammatory cytokines such as TNF-alpha and immunosuppression with corticosteroids. Such a mechanism might contribute to the frequent detection of HHV-6B reactivation in patients diagnosed with Drug Reaction with Eosinophilia and Systemic Symptom (DRESS), a potential fatal syndrome initiated by adverse drug reactions [61,62].

Understanding the functional changes described above will be enhanced by deep analysis of the effects of roseolovirus infection on host cell transcription, translation, and export of gene products.

RESEARCH PRIORITIES

Understanding of the molecular virology of roseoloviruses lags behind that for all other human herpesviruses. Understanding the genetic content of roseoloviruses has not been extended far beyond basic sequence analysis of laboratory-adapted strains. Modern methods of DNA sequencing need to be applied to understanding the sequence composition of wild, uncultured roseolovirus genomes, as well as inter- and intrahost sequence variation at the genome level in immune competent and immune compromised individuals. Among other things, such genetic analyses are necessary to ensure that animal studies and other experiments are done with viruses that appropriately represent wild viruses. Functional analysis of the the genes unique to roseoloviruses and betaherpesviruses will provide information as to how these viruses have adapted to their specific and specialized niches. Genetic approaches using BAC-based recombination strategies are critical to identify the viral factors and cis-determinants of replication, integration, and reactivation. Even in the absence of well-established genetically tractable systems for HHV-6B and HHV-7, transcript and proteomic profiles can rapidly confirm putative genes and identify novel ORFs, novel transcript forms, and non-coding RNAs. Vaccine development typically involves attenuation, but intelligently designed attenuation will not be possible for the roseoloviruses without fundamental knowledge of replication and host interaction determinants.

SUMMARY

Roseolovirues have unique cellular tropisms and biological properties, and encode ORFs distinct from the other human betaherpesvirus, HCMV. Each HHV-7 gene has a homolog in HHV-6A and HHV-6B. However, HHV-6A and HHV-6B have several genes not found in HHV-7, including a homolog of the parvovirus rep protein, U94. Roseolovirus gene products mediate cell entry and viral replication, modulate the host cell's growth, survival, signaling, and immune responses, and regulate latency. RNA analyses and proteomics coupled with new genetic tools and advances in systems biology are needed to advance the identification and function of known, as well as uncharacterized and novel ORFs, and transcripts such as miRNAs. Advancements in understanding roseolovirus gene function will reveal novel virus-host interactions and better define the mechanism of integration and excision of the virus genome into and from host chromosomes, a potential form of latency that would be unique among the human herpesviruses. Investments in understanding the fundamental molecular processes of roseolovirus infections will inform our understanding of the dynamic process of persistence and disease in humans and identify targets for therapeutic intervention.

ACKNOWLEDGEMENTS

L.T.K. was supported by an American Cancer Society Research Scholar Grant RSG-11-160-01-MPC and NIH AI111129-01 and AI097875-01.

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Highlights

- HHV-6A, HHV-6B, and HHV-7 are distinct members of the Roseolovirus genus.
- HHV-6A and HHV-6B can integrate into the telomeres of the host chromosome.
- Lytic infection involves regulated expression of viral proteins and non-coding RNAs.
- Infection alters cellular processes, and innate and adaptive immune responses.
- Many aspects of their molecular biology remain to be defined.

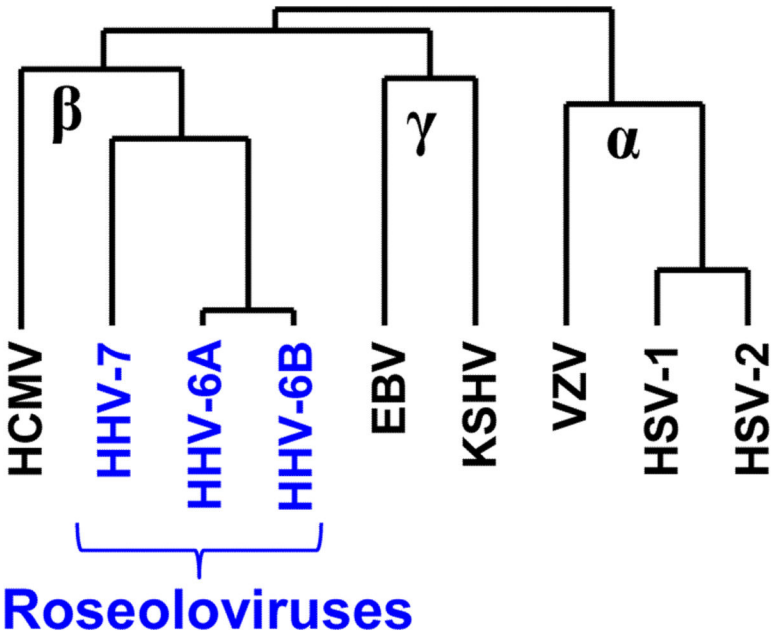

Figure 1.
Dendrogram showing relationships among the human herpesviruses, based on sequences of the conserved protein, gB.

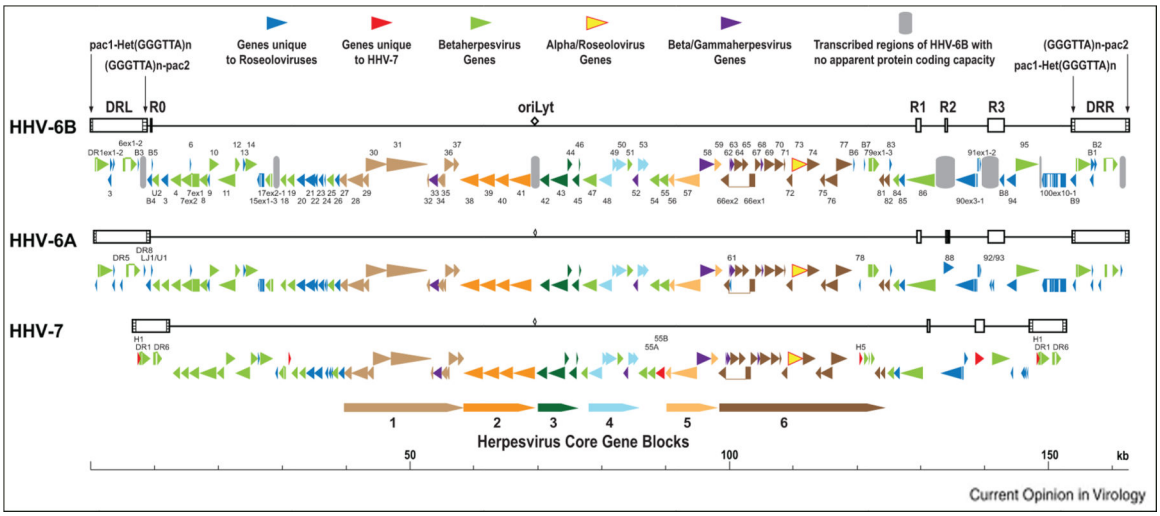

Figure 2.

Genomic and genetic architectures of the human roseoloviruses. Based on information from [15,63-67].

Table 1

Genetic and biological properties of human roseoloviruses and HCMV.

	HHV-6A	HHV-6B	HHV-7	HCMV
Commonly used strains	U1102, GS	Z29, HST	JI, RK, SB, UCL-1	AD169, Towne, Merlin, TB40E
Length of wild genomes	?	~170 kb	?	236 kb
Length of passaged genomes	159 kb	159-162 kb	145 kb	~230 kb
genes encoding unique proteins	~102	~97	~86	~165
miRNAs	4 predicted	4	unknown	16
Replication	slow, extended			
	ballooning, refractile cytopathic effect			cytomegaly, nuclear and cytoplasmic inclusions
	origin-binding protein for initiation of DNA replication			
Cell surface receptor	CD46	CD134	CD4	EGFR, Integrins
Cell culture tropism	umbilical cord blood lymphocytes peripheral blood mononuclear cells			monocyte-macrophages
	T cell lines: SupT-1, HSB2, J JAHN	T cell lines: Molt-3, Mt-4, SupT-1	T cell lines: SupT-1	CD34+ hematopoietic cells
	productive replication in astrocytes	low-level persistence in astrocytes		endothelial and epithelial cells, fibroblasts
Unique features	integration into host telomeres			
Major disease associations	Hashimoto's thyroiditis	exanthem subitum		congenital birth defects
		febrile seizures/ status epilepticus		transplant complications
		transplant complications		retinitis
		post-transplant reactivation-associated encephalitis		hepatitis

Table 2

Genes unique to roseoloviruses.

Function[a]	Roseolovirus ORF	%S with HHV-6A[b]	% I with HHV-6A[c]	%S with HHV-7	%I with HHV-7
Roseolovirus specific genes					
	U13	93.4	92.5	44.9	35.7
	U15EX1	91.4	86.7	76.4	67.9
	U15EX2	100	95.8	83.3	75
	U15EX3	96.7	91.7	82	75.4
Glycoprotein	U20	95.6	95.6	31.8	22.2
Down-regulation of MHC class I	U21	91	89.8	42.8	31.6
Glycoprotein	U23	94.6	94.1	26.9	20.9
	U24	88.3	82.7	46.2	31.2
	U24A	94.7	91.2	40.3	28.1
	U26	93.8	92.9	60.5	47.4
OX-2 homology, glycoprotein	U85	93.1	91.7	46.9	36.8
IE-A (IE1), transactivator	U90EX1	73.7	68.4	42.8	35.7
	U90EX2	70.3	67.2	67.1	57.1
	U90EX3	76.7	71.5	32.9	25.2
IE-A	U91EX1	67.8	57.1	33.3	25
	U91EX2	69.2	67.9	45.6	40
Spliced envelope glycoprotein; HHV-6 pg82-gp105, HHV-7gp65	U100EX1	78.1	73.4	27.2	19.7
	U100EX2	84.9	81.7	53.8	38.7
	U100EX3	82.9	79.3	40.9	32.7
	U100EX4	96	88	44	40
	U100EX5	88.6	80	34.3	28.6
	U100EX6	91.9	91.9	48.6	37.8
	U100EX7	88.7	83	35.3	27.4
	U100EX8	100	100		
	U100EX9	92.8	90.5	35.7	23.8
	U100EX10	83.9	76.5	24	13.3
HHV-6 specific genes					
	DR3	87	86.4		
	U6	97.1	97.1		
	U9	94.2	94.2		
Glycoprotein	U22	91.2	89.6		
Intercrine cytokine	U83	87.6	85.6		
Parvovirus rep homolog	U94	98.4	97.6		
HHV-6A gene					

Function[a]	**Roseolovirus ORF**	**%S with HHV-6A**[b]	**% I with HHV-6A**[c]	**%S with HHV-7**	**%I with HHV-7**
	U78				
HHV-6B genes					
	B3,B4,B5,B6,B7,B8				

[a] Implied functions of homologous genes or experimental validation.

[b] Percentage of amino acid similarity between homologs.

[c] Percentage of amino acid identity between homologs.