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MOLECULAR MECHANISMS OF VIRUS SPREAD AND VIRION COMPONENTS AS TOOLS OF VIRULENCE

A REVIEW*

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Despite of differences in replication strategy among virus families, some basic principles have remained similar. Analogous mechanisms govern virus entry into cells and the use of enzymes which direct the replication of the virus genome. The function of many cell surface receptors (such as glycosoaminoglycans, glycoproteins, proteins) which interact with viral capsid proteins or envelope glycoproteins has recently been elucidated. The list of cellular receptors (Table I) is still far from being final. The capsid components, similarly as the envelope glycoproteins, may form specific pocket like sites, which interact with the cell surface receptors. Neutralizing antibodies usually react with antigenic domains adjacent to the receptor binding site(s) and hamper the close contact inevitable for virion attachment. In the case of more complex viruses, such as herpes simplex virus, different viral glycoproteins interact with several cellular receptors. At progressed phase of adsorption the virions are engulfed into endocytic vesicles and the virion fusion domain(s) become(s) activated. The outer capsid components of reoviruses which participate in adsorption and fusion may get activated already in the lumen of digestive tract, i.e. before their engulfement by resorptive epithelium cells. Activation of the hydrophobic fusion domain(s) is a further important step allowing to pass through the lipid bilayer when penetrating the cell membrane in order to reach the cytosol. Activation of the virion fusion domain is accomplished by a conformation change, which occurs at acid pH (influenza virus hemagglutinin, σ1 protein of the reovirus particle) and/or after protease treatment. The herpes simplex virus fusion factors (gD and gH) undergo conformation changes by a pH-independent mechanism triggered due to interaction with the cell surface receptor(s) and mediated by mutual interactions with the viral envelope glycoproteins. The virion capsid or envelope components participating in the entry and membrane fusion are not the only tools of

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virulence. The correct function of virus coded proteins, which participate in replication of the viral genome, and/or in the supply of necessary nucleotides, may be very essential. In the case of enteroviruses, which RNA ineracts with ribosomes directly, the correct configuration of the non-coding viral RNA sequence is crucial for initiation of translation occurring in the absence of the classical "cap" structure.

Keywords: virulence of viruses, myxoviruses, enteroviruses, lyssavirus, reoviruses, herpes simplex viruses

Viruses infecting the human and/or animal body must overcome the natural anatomic barriers and resist the inactivating (inhibitor) substances already at first contact in the portal of entry. Viruses enter the body mainly via mucous membranes and/or by skin. These tissues are in abundance equipped with cells mounting nonspecific as well as specific immune responses. The mucus secreting and IgA producing goblet cells cover the mucosa of nasal turbinates, trachea, bronchi, bronchioli, stomach and of large intestine. In the respiratory tract, the movement of ciliary epithelium removes particles and air droplets larger than 5 μm, while smaller ones become engulfed by alveolar macrophages. The latter then move to regional lymph nodes to present antigenic peptides and to elicit immune responses. Viruses which enter the body via the arthropode bite are either directly deposited into bloodstream or they enter the skin lymphatics. In the former case they still may be cleared by scavenger cells (in the spleen red pulp) or by Kupfer cells in liver. In the latter case they are ingested mainly by connective tissue histiocytes and epidermal dendritic cells and, once again, transported into regional lymph nodes.

Viruses developed many tools to overcome these obstacles especially at early stages of infection. Virulent virus strains are able to replicate quickly and efficiently at the portal of entry and possess tools supporting their spread to target organs (such as the central nervous system, CNS). The most crucial property related to virulence, is the ability of virus particles to interact with the surface of susceptible target, mediator and/or transporter cells. The process of adsorption and penetration, which has been profoundly investigated in cell culture, leads to liberation of the viral genome into cytoplasm (in some cases followed by the genome transport into nucleus). This is usually followed by initiation of the virus replication cycle (establishment of latent infection might be an exception). Some viruses replicate in the cells of immune system such as T- or B-lymphocytes, macrophages and/or Kupfer cells. An efficient way to reach the target organ such as CNS, resides in the ability of some viruses to adsorb to the nerve endings and to use the quick axonal transport for spreading to the body of neurons.

Table I

Important virus receptors*

Family	Virus	Receptor	Reference
Adenoviridae	Adenovirus 2	Integrins $\alpha_v\beta_3$ and $\alpha_v\beta_5$	Wickham et al. [113]
Coronaviridae	Mouse hepatitis virus	Glycoproteins from the family of carcinoembryonic antigens	Yokomori and Lai [114] Dveskler et al. [115]
	Human coronavirus 229E	Aminopeptidase N	Yeager et al. [116]
Herpesviridae	Epstein-Barr virus	CD21 (B-lymphocytes) receptor; HLA class II glycoproteins	Nemerow et al. [117] Fingeroth et al. [118] McClure et al. [119]
	Herpes simplex virus	Glycosoaminoglycans and HVEMs (herpes virus entry mediators: TNF and Ig receptor family proteins)	Shich et al. [120] Spear [121] Campadelli-Fiume et al. [122]
	Cytomegalovirus	Glycosoaminoglycans and the HLA class I receptor subunit (β_2-microglobulin)	Grundy et al. [123] Compton et al. [124] Wykes et al. [125]
	HHV6 and 7	CD4 receptor (T-lymphocytes)	
Ortho-myxoviridae	Influenza virus A, B	Sialic adic residues	Higa et al. [126] Weiss [127]
Picornaviridae	Rhinovirus	Intercellular adhesion molecule (ICAM-1)	Greve et al. [128] Staunton et al. [129]
	Poliovirus	Poliovirus cell receptor (Ig superfamily)	Mendelsohn et al. [17]
	Echovirus 1 and 8	VLA-2, α-2 integrin (CD49b), very late antigens on lymphocytes and monocytes	Bergelson et al. [130]
Poxviridae	Vaccinia virus	Epidermal growth factor (EGF) receptor	Epstein et al. [131]
Reoviridae	Reovirus 3 (Dearing)	Sialic acid residues EGF receptor	Gentsch and Pacitti [69] Paul et al. [70] Tang et al. [71]
Retroviridae	Human immuno-deficiency virus (HIV)	CD4 receptor (T-lymphocytes)	Dalgleish et al. [132] Klatzman et al. [133]
	Human T cell leukemia virus		Sattentau and Weiss [134] Weiss [135]
		Galactosylceramid	Bhat et al. [136]
Rhabdoviridae	Rabies virus	Acetylcholine receptor	Burrage et al. [57] Lentz [137] Hahnham et al. [138]
	Vesicular stomatitis virus	Gangliosides and phospholipids	Superti et al. [139] Schlegel et al. [140]
Togaviridae	Sindbis virus Semliki Forest virus	Laminin receptor HLA class 1 molecules (in mice H2-K and H2-D)	Wang et al. [141] Helenius et al. [142]

* according to Tyler and Fields [89].

Clearly, cellular receptors, which interact with the virion surface components, fulfil other important physiological or biological functions, i.e. there are not in order to act as virion receptors. Table I shows examples of some virus receptors, which have been identified so far. Many of them were discovered by means of well defined ligands, which are able to interfere with virus adsorption and prevent infection.

An important feature of myxoviruses is their ability to replicate at higher temparature, i.e. during fever. In the case of influenza virus, this property is probably related to the function of the viral RNA polymerase complex. Strains, which had been adapted to 34 ºC and do not replicate at 37 or 39 ºC, behave as attenuated and would not cause disease in monkeys and man [1]. Alternatively, rhinoviruses (cause sneezing) grow better at 34 ºC than at 37 ºC. Because the temperature of nasal turbinates is close to 34 ºC, rhinoviruses never cause bronchitis and always remain restricted to the upper respiratory tract. In addition, these viruses are acid labile (despite of the absence of an envelope). They undergo complete inactivation at pH 3 within 30 minutes and are highly sensitive to pH 6 treatment [2]. In contrast, enteroviruses, members of an another genus of the *Picornaviridae* family, are not only resistant to lower pH, but also resist to duodenal juice containing bile salts and several proteases. The relative resistance of enteroviruses against lower chlorine concentrations depends widely on the presence of other organic substances and is of wide practical importance [3].

The members of Enterovirus genus from the *Picornaviridae* family (72 serotypes, several important human pathogens such as poliovirus and hepatitis A virus) are small non-enveloped capsids containing single stranded RNA of positive polarity, which is capable to interact with cytoplasmic ribosomes. These viruses enter the body via intestinal tract mucosa by adsorbtion to M cells and/or by crossing the columnar resorptive epithelium (brush border cells). Enteroviruses do not replicate in the intestinal surface epithelium cells, but rather do so in the Payer patches of submucosa and in the regional mesenteric lymph nodes. Furthermore, polioviruses can start to replicate in tonsils when crossing the nasopharyngeal squamous epithelium; then the poliovirus (especially in association with tonsillectomy) may quickly enter the nerve endings and can reach the brain stem [4]. Nevertheless, viraemic spread is the traditionally accepted route, how poliovirus invades the CNS in order to involve the large motoneurons of the anterior horns in spinal cord causing their destruction [5].

The picornavirus RNA which has about 7500 nucleotides (up to 8000 nts in other enteroviruses) codes for a large polyprotein; this is later on cleaved by self digestion. The 5'-end portion of the polyprotein yields the capsid proteins VP1,

VP3 and the VP0 precursor polypeptide; the latter is finally split into VP2 and VP4 capsid proteins. The 3'-end of viral RNA (vRNA) codes for the viral RNA polymerase and for additional non-structural polypeptides with less well defined functions [6]. Comparison of the original (P1/Mahoney/41) poliovirus type 1 RNA sequences with the sequence of the attenuated poliovirus vaccine strains (P1/Sabin) has revealed up to 50 mutations, from which about 10 seem up most important (Table II). The attenuated phenotype of above-mentioned mutations was demonstrated in transgenic mice expressing the human poliovirus receptor [7]. Surprisingly, studies in monkeys did not confirm the overall importance of mutations at G-480 and C-6203 [8]. Nevertheless, the role of A-480 and U-525 for neurovirulence has been confirmed by sequencing of revertants causing vaccine-associated paralytic poliomyelitis [9]. The above-mentioned mutations in the 5′-ncr (non coding region) are important for correct folding of the vRNA in the region called

Table II

Most frequent mutations in the vRNA of attenuated strain P1/Sabin*

Nucleotide		Region	Amino acid	
No.	Mutation		No.	Mutation
Nt 21	U to C	5-ncr		
Nt 189	C to U	5-ncr		
Nt 480	A to G**	5-ncr		
Nt 525	U to C**	5-ncr		
Nt 935	G to U	VP4	Aa 65	Ala to Ser
Nt 2438	U to A	VP3	Aa 235	Leu to Met
Nt 2795	G to A	VP1	Aa 106	Ala to Thr
Nt 2879	C to U	VP1	Aa 134**	Leu to Phe
Nt 6203	U to C**	RNApol	Aa 73**	Tyr to His
Nt 7071	G to U	RNApol	Aa 362	Met to Ile
Nt 7441	G to A	3' -ncr		

* according to Horie et al. [7] and Friedrich [11, 12]
** very important mutations
ncr = non-coding region
note: the second nucleotide or amino acid is the mutated phenotype

IRES (internal ribosomal entry site). This vRNA region interacts with the 40S subunit of ribosomes. Foldings of vRNA are important for forming the active translation complex and function after interaction with cellular helper proteins in the presence of the *e*ukaryotic (translation) *i*nitiation *f*actor (eIF). Picornavirus RNA lacks the 5'-end m^7G cap, the traditional hallmark of the eukaryotic mRNA. It is believed that correct folding of the vRNA at IRES bypasses the use of the classical m^7G cap. In addition, an internal picornavirus polyprotein protease activity

(polyprotein region 2A, which cleaves the polyprotein to form the capsid proteins, to split off the main subunit of viral RNA polymerase and its additional cofactors), destroys also the cellular *c*ap-*b*inding *c*omplex (CBC) needed for translation of cellular mRNAs. Thus, picornaviruses are able to avoid the cap-binding system to eliminate the translation of cellular mRNA (in order to stop the cellular proteosynthesis) and, they can initiate the translation of their own RNA by means of an unic IRES within the vRNA [10]. It should be stressed here that reverting mutations at non coding nts 480 (G to A for P1 Sabin), 481 (A to G for P2 Sabin) and 472 (U to C for P3 Sabin) were detected in almost each revertant strain isolated in vaccine-associated paralytic poliomyelitis [11, 12].

Further mutations, influencing picornavirus virulence, are in vRNA regions encoding the RNA polymerase and the capsid proteins, respectively. VP1, the major capsid protein is essential part of the protomer, the virion subunit formed by proteins VP1, VP2, VP3 and VP4 [13]. The capsid proteins form a surface groove representing the site of interaction with cellular receptors such as ICAM-1 and ICAM-2 (intercellular adhesion molecule) or VCAM (vascular adhesion molecule) [14, 15]. ECHO viruses interact with the leucocyte *v*ery *l*ate *a*ntigen VLA-2 (integrin $\alpha 2$) and with the complement activation regulating decay accelerating factor (DAF or CD55) present in the leucocyte membranes [16]. Other receptors which interact with enteroviruses are the leucocyte adhesion factor LFA-1 (*l*eucocyte *f*unction *a*ssociated), the LDL-receptor and/or the cell surface sialoglycoproteins. The poliovirus itself interacts with a functionally less well defined integrin called PVR (*p*olio*v*irus *r*eceptor) belonging to the immunoglobulin gene super-family [17]. Virus-neutralizing antibodies bind to several antigenic domains of VP polypeptides mainly at the sites surrounding the receptor-binding groove. Rhomadine or Arildon derivatives, the so-called WIN compounds, are specific antagonists reacting with the groove-forming domain of VP1 [18]. The amino acid sequence motif RDG at residues 145–147 and an adhesion peptide at aa 203–213 have been implicated as receptor binding sites for the foot-and-mouth disease virus VP1 (Aphthovirus) [19]. Following receptor binding, a conformation change occurs in VP1 creating a transmembrane channel through the lipid bilayer, which still surrounds the virus particle located in the pinocytic vacuole or pit. During this penetration process, also called receptor mediated endocytosis, putative hydrophobic protrusions of VP1 and VP4 capsid proteins might form a channel enabling the RNA genome to pass through the membrane. The unfolding of the hydrophobic peptide domains is trigerred by acidification of the pinocytic vesicle content brought about by an ATPase dependent proton pump [20].

Even more convincingly, similar principles can be demonstrated for influenza virus entry (family *Orthomyxoviridae*), which probably represents the most typical model for pH dependent penetration (Figure 1). Unlike to picornaviruses, orthomyxoviruses show the helical symmetry; their helix is formed by the ribonucleoprotein (RNP), which contains the vRNA and many (over 1000 copies) nucleoprotein (NP) subunits. The influenza virus RNA is single stranded, segmented and reveals negative polarity, i.e. it cannot be directly translated. The virus carries its own RNA polymerase, a protein complex which either transcribes the mRNAs or can copy a full length complementary (intermediate) RNA, which becomes the template for new vRNA synthesis [21]. The viral RNA polymerase complex consists of three proteins termed PB1, PB2 and PA (Figure 2A), from which PB2 has a cap-binding activity and PB1 an endonuclease activity [22, 23]. The short oligonucleotides (m^7GpppNm) which initiate viral mRNA transcription are of cell origin [24]. The viral endonuclease cleaves the cellular mRNAs to generate these primers [25]. Mutations in the PB2 subunit protein reduce the pathogenicity of influenza A virus by hampering viral mRNA transcription. Another mutations in the viral RNA polymerase complex are related to the loss of the ability of low pathogenic (attenuated) strains to replicate at 37 ºC. The cold adapted influenza strains would replicate well at 34 ºC, but not at 37 ºC, while the highly virulent strains would still replicate at 39 ºC. When the viral RNA polymerase subunit

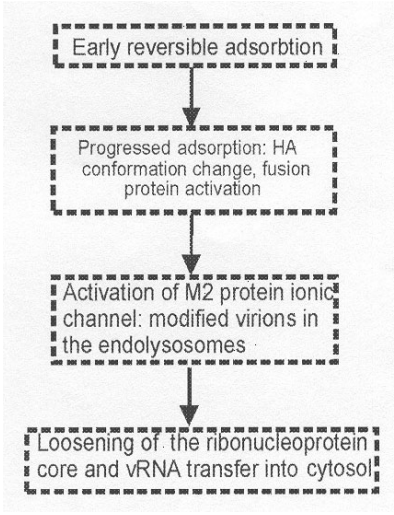

Figure 1. Schematic representation of early events at influenza virus adsorption and penetration. The events may be similar at any other pH dependent penetration, when acid pH triggers fusion domain activation

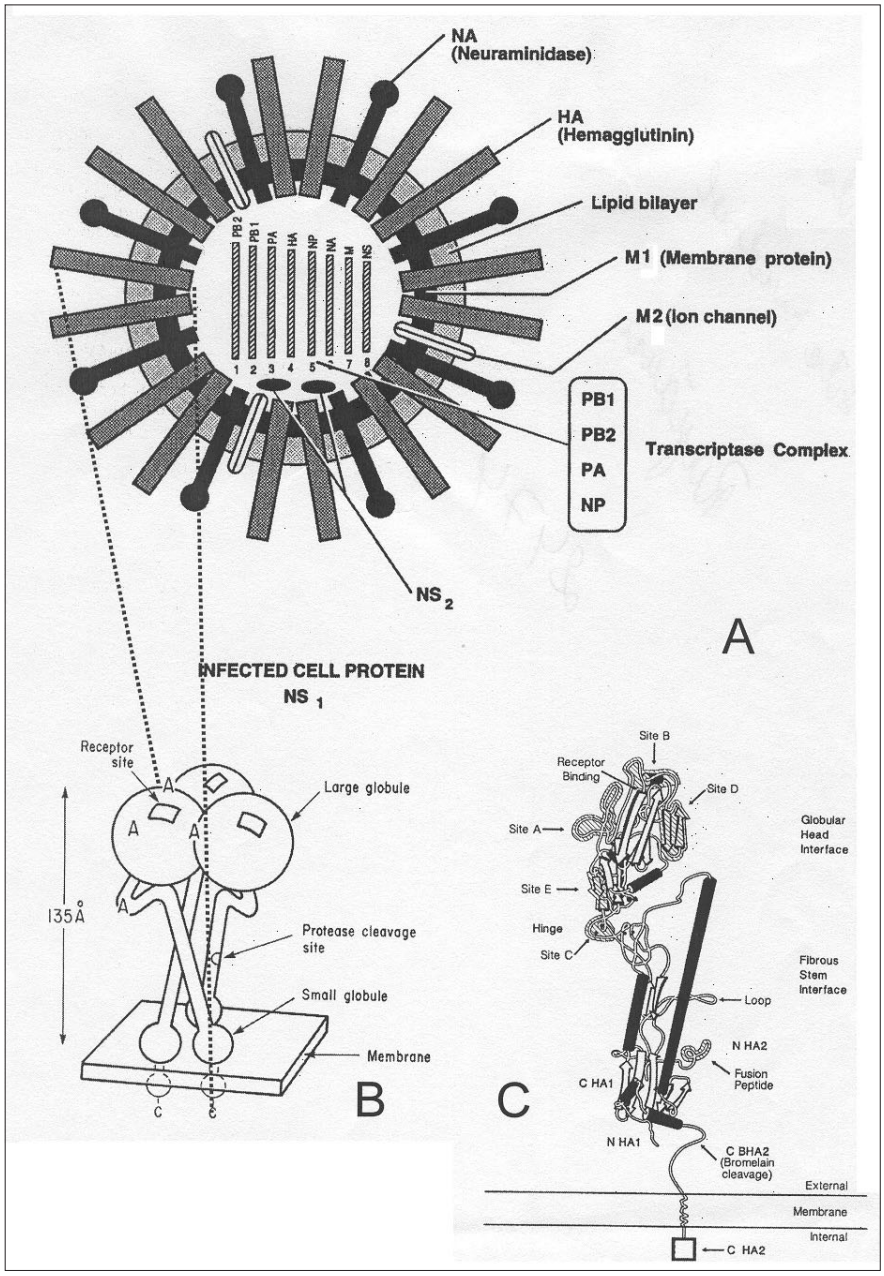

Figure 2. The influenza virus particle (A) with the HA trimer enlarge (B) and the tertiary structure of HA (C) showing the position of antigenic domains (sites A-E) and of the HA2 subunit fusion peptide (at 2C in the right). For details see text (modified according to Lamb and Krug [22] and Murphy and Webster [37])

proteins interact with the newly synthesized viral NP (not yet bound into RNP), the activity of this modified complex terminates viral mRNA synthesis and starts copying the full length intermediate RNA. The modified RNA polymerase complex also participates in the synthesis of vRNA [26, 27]. Important regulatory function is fulfilled by the virus coded non-structural NS1 protein, which inhibits polyadenylation and transport of cellular mRNAs from nucleus to cytoplasm [28].

In contrast to above-mentioned viral proteins, the envelope glycoprotein polypeptides (HA = hemagglutinin, NA = neuraminidase) are synthesized in the rough endoplasmic reticulum and further glycosylated in the Golgi apparatus. The HA0 polypeptide, which is the orginally translated HA gene product (after splitting off the 16 aa signal sequence) has 566–576 aa depending of the HA serotype [29]. In the Golgi zone it is cleaved into HA1 and HA2 chains by a trypsin-like enzyme and an exopeptidase. The HA2 chain (220–221 aa) remains inserted within the lipid bilayer by a transmembrane domain, while the HA1 chain (317–328) gets joined with it by a bisulfidic bond only [30, 31]. The HA molecule present in the spike of the virion envelope is a homotrimer (Figure 2B) consisting of three HA1/HA2 pairs [32]. Though there is no specific function for glycosylation, it is believed that the carbohydrate moieties help at correct folding and keeping the trimer subunits together in optimal conformation [33]. The HA0 cleavage site has a conservative sequence with a single Arg, mutation of which would render the virus non-infectious [34, 35]. Cell lines lacking the suitable peptidase would also produce non-infectious particles [36]; in such case, trypsin digestion may restore virulence [37].

The exopeptidase cleavage of HA2 creates a new N-terminus, which has an hydrophobic domain masked within the HA trimer structure [38]. This "hidden" fusion domain becomes active after the pH-dependent conformation change of the trimer molecule [39, 40]. Before the conformation change occurs, the HA2 fusion domain has a relatively distant position from the globular head of HA molecule, where the receptor-binding pit is located. At low pH confirmation, the HA2 residues 40–105 form a triple-stranded 10 nm long α-helical coil (instead of the α-helix between aa 76–126), while residues 106-112 uncoil to form an extended loop. Finally, the membrane proximal region swings up against the long coil [41, 42]. The conformation change of HA usually occurs within the endocytic vesicle at progressed stage of adsorption.

The receptor-binding pocket of the HA molecule is formed by several folds of the HA1 subunit with 5 essential amino acids (Tyr 98, Trp153, His183, Glu190 and Leu194) [41, 43] which mutations would decrease infectivity and impair the fit between the receptor binding pocket and the cellular receptor. The latter is sialic

acid [44] linked to galactose or galactosamine which are part of the glycosoaminoglycan (GAG) surface. The binding of sialic acid to the HA pocket could be abolished by a single mutation at the HA2 residue 226 [45]. Noteworthy, the receptor binding locus is not the site, where the neutralizing and hemagglutination inhibiting antibodies bind. Rather, the antibodies interact with several epitopes (A to E, Figure 2C) surrounding the receptor binding site located at the globular head formed by the HA1 subunit. An important factor of influenza virus virulence was traditionally attributed to the variability of HA and NA antigenic domains. Single mutations at these sites (antigenic drifts) may impair the binding of antibodies. However, the emergence of a completely different antigenic type of HA and/or NA (antigenic shift) would considerably abolish the antibody binding. The antigenic shift leading to appearance of a new HA molecule occurs due to reassortment (genetic exchange of the vRNA segment 4 between two different influenza virus strains) during double infection in an animal (avian or other) reservoir [46, 47]. By such mechanism, the new extremely virulent H3N2 strains started to circulate during the last two decades, and more recently, the virulent H1N1 reappeared as soon as the majority of current population ceased to reveal the corresponding antibodies.

The recent hypothesis of influenza virus penetration into susceptible cells can be summarized as follows [48]. The enveloped influenza virus particles attach to the ciliary columnar epithelium cells in respiratory airways after crossing the mucus layer by the help of viral NA activity. The receptor binding site of envelope spike (HA trimer) interacts with the sialic acid moiety of GAG at epithelium surface. After attachment, acidification of the endocytic vesicle content (fusion with a lysosome) is of essential importance. At pH lower than 6, the above described conformation change brings the HA2 hydrophobic domain into close vicinity of the globular region of the HA1 subunit. The HA2 fusion domain intercalates the lipid double membrane and loosens the wall of endocytic vesicle. This event is correlated with the action of M2 protein. The latter protein forms a tetramer penetrating across the lipid bilayer of the influenza virus envelope [49]. Acid pH activates the ion channel domain of M2 protein transporting hydrogenium ions and other kations into the virion core. The ionic influx helps to liberate the vRNA from RNP forming the core complex. This function of M2 protein can be inhibited by Amantadine [50, 51]. Summing up, the acid pH within the endocytic vesicle activates the fusion domain of HA molecule and triggers the influx of cations into viral particle in order to loosen the binding between NP and vRNA and to liberate the latter. Finally, the vRNA is transported into nucleus of infected cell in order to start the transcription of viral mRNA.

Briefly, the tools of influenza virus virulence such as hemagglutinin and the proteins of viral RNA polymerase allow the penetration and subsequent replication of viral particles in the columnar epithelium cells of respiratory mucosa close to the portal of entry site. Secondary spread to distant organs is a rare event. Unfrequent mutations at conservative HA sites (cleavage site, receptor binding pocket) impair the functional properties of HA. Alternatively, also mutations in the components of viral RNA polymerase complex, may decrease virulence. On the other hand, the frequent mutations, which change the antigenic properties of variable sites of the HA molecule, can impair antibody binding and increase the influenza virus pathogenicity just slightly. However, the feasibility of reassortment, allowing replacement of segment 4 gene encoding the recently circulating HA by a new or a former circulating HA gene, yields an efficient new tool extremely increasing influenza virus virulence.

A classical example of viral spread along nerves to the CNS is the intraaxonal transmission of rabies virus. The lyssavirus *(Rhabdoviridae)* has a simple structure and consists of five proteins [52]. The main tool of virulence here is a single glycoprotein, the G protein, which, similarly to influenza virus HA, forms a homotrimer. Its precursor polypeptide, after splitting off the signal sequence, usually has 505 amino acids, though the actual number of amino acids may vary according to rabies virus serotype [53]. The rabies virus particle contains a single stranded vRNA of negative polarity, which associates with a nucleoprotein (N protein). The bullet shaped particle has an internal membrane (M) protein being surrounded with a lipid envelope containing the G protein. In addition, the virus particle carries its own RNA polymerase consisting of a large (L protein) and a small subunit (phosphoprotein, P protein). The rabies virus particles show high affinity to naked nerve endings in the skin or cornea as well as to neuromuscular nerve end-plates in skeletal striated muscles and/or to neurotendinal spindles thereof [54, 55]. This property seems entirely related to G protein. Its adsorption to nerve endings can be prevented by β-bungarotoxin and/or δ-tubocurarine. Both toxins block the interaction of rabies virus G protein with the nicotinic acetylcholine (ace) receptor at the above-mentioned nerve endings [56, 57, 58]. Under physiological conditions, the ace-receptor either at the nerve endings or at the synapses mediates motoric and/or sensoric signaling. The G protein has a peptide domain (between aa 172–202), which is nearly identical with a similar domain within the bungarotoxin molecule. The invasivity of G protein into nerve endings was found related also to Arg333 of the G protein polypeptide [59] and to an additional residue located in its antigenic domain III [60]. The G protein, furthermore, interacts with receptors on non-neural cells, probably with a membrane phospholipid receptor, sensitive to

phospholipase action. The mechanism of rabies virus penetration was not fully elucidated, but is probably similar to that of the pH-dependent HA-mediated membrane fusion. Finally, the G protein has a hydrophobic (fusion) domain, which upon activation, initiates membrane fusion and giant cell formation [61, 62]. Its precise location was not identified, but according to the expression of an analogous vesicular stomatitis virus (VSV) G protein, the position of such domain was located between aa 118–136. After entering the nerve endings, the rabies RNP complex is transported by quick axonal transport [63]. Similarly as in case of herpes viruses, the rabies virus RNP complex probably moves along microtubules [64].

Interesting data became evident when summarizing the studies on molecular pathogenesis of reovirus and/or rotavirus infections (family *Reoviridae*). The non-enveloped reovirus particles contain double stranded (ds) RNA arranged into 10 segments, which were classified according to their electrophoretic mobility as small (S segments), medium (M segments) and large (L segments). The most important tools of virulence are the outer capsid proteins σ1, σ3 and μ1 (encoded by segments S1, S4 and M2, respectively) and the spike protein λ2 [65]. Protein σ1 plays important role in virion attachment and hemagglutination [66]. It also determines the tropism mediated by cell receptors and the interaction with axonal microtubules during axonal transport occurring with some reovirus strains [67, 68]. This immune dominant protein has several antigenic domains reacting with neutralizing antibodies and a domain inducing T-cell mediated cytotoxic response. The receptors for σ1 are mainly sialoglycoproteins [69, 70]. Attachment to the non-neural epidermal growth factor (EGF) receptor has also been reported [71]. The data on interaction with the neural β-adrenergic receptor are tempting but controversial [72, 73, 74]. While binding of σ1 from strain T3D to sialic acid receptor maps to residues 194–204 [75], the interaction with the EGF receptor was localized to aa 317–332. The interaction of σ1 protein with nerve endings was related to a domain between aa 340–419.

Protein σ3 is a zinc-finger metalloprotein which binds to dsRNA, regulates viral mRNA transcription and translation and inhibits cellular proteosynthesis [76, 77, 78, 79]. This protein also interacts with μ1 present in the intact virion, but not with the cleaved μ1C, which already underwent protease treatment and activation by an acid pH environment [80]. It has been known since many years that Reo- and Rotaviruses become activated by acid pH and duodenal proteases being converted into so-called infectious subviral particles (ISVP) [81, 82]. Thus, at least two essential cleavage events take place before the capsids get ready for penetration, namely the removal of σ3 protein and the digestion of μ1 into μ1N (the

myristoylated N-terminus) and μ1C (the C-terminus of μ1 outer capsid protein). The latter polypeptide (μ1C) finally splits into fragments δ and φ [83]. In addition to the absence of σ3 protein and formation of the stable fragment δ (generated from μ1C) the main hallmark of ISVP is the conformation change of the σ1 protein needed for penetration [84]. The ISVP may be generated in the gastrointestinal tract when the virus passes through the stomach and duodenum of infected mice in order to reach the small intestine [85]. A current model suggests, however, that ISVP can be generated in endolysosomes. Little is known about virion penetration into cytosol, but the conformation change of the above-mentioned outer capsid components is certainly of great importance [86]. In rotaviruses, the function of protein μ1/μ1C has been attributed to capsid protein VP4, which is cleaved into components VP8 and VP5 by a similar mechanism, i.e. in an acid environment and in the presence of pancreatic proteases [87]. The last conformation change, characteristic for the cytoplasmic core particles is the conformation change of λ2 protein (activation of viral guanyltransferase) which initiates transcription together with the virion RNA polymerase.

The experimental pathogenesis of reovirus infection in mice has shown that strain T1 Lang (T1L) and T3 Dearing (T3D) differ strongly in their spread in the body and secondary distribution. Strain T1L after oral inoculation grows to high titers in intestinal tissue when involving the columnar epithelium cells and the M cells of small intestine. Then it spreads to local lymph nodes, causes viraemia and invades the liver and spleen. Finally, in the course of secondary viraemia, T1L virus infects the meninges, namely after crossing the plexus choriodeus it reaches the ependyma cells. In contrast, strain T3D multiplies in the intestinal wall to relatively lower titers and spreads along axons to reach the neurons in the gray matter of CNS. The ability of strain T3D to invade the neuromuscular tangles and striated muscle motoric end plates (similarly to rabies virus) was confirmed by intramuscular inoculation of mice [88]. Clearly, this different behaviour of above-mentioned reovirus strains has been attributed to the properties of σ1 protein encoded by the corresponding S1 gene [89].

Herpes simplex viruses 1 and 2 (HSV-1 and HSV-2) are ubiquitous pathogens which may reside latent in the regional sensory ganglia. Relatively rarely, life threatening complications occur, such as generalized diseases in newborn and encephalitis in adults. The genome of HSV is a dsDNA with internal and external repetitions flanking a long and short unic segment. The HSV DNA codes for over 80 genes, from which 66 are localized in the unique long (UL) segment and 14 in the unique short (US) segment. Further 2 genes, are located in the repetitive sequences *b* and *c* (together 4 ORFs) flanking the UL segment, at least 1 gene is lo-

cated in the repetitions flanking the US segment. The total number of genes, however, is still not final, because additional ORFs, such as *P* and *O*, were found in the complementary DNA strand. Furthermore, at least two putative proteins may be coded by the ORFs which are present in the latency associated transcripts, permanently transcribed during latency [90, 91,92].

At least nine of the eleven HSV-1 glycoproteins have been characterized as regards their role in the virus replication cycle (Table III). Briefly, at early adsorption, the gC interacts at the cell surface with the haparansulphate moiety of GAGs. Additional 4 HSV envelope glycoproteins participate in progressed stages of adsorption mediating a pH independent penetration, namely gB, gD and the gH/gL heterodimer. Glycoprotein B has an alternative GAG binding domain and regulates membrane fusion by its C-terminus (UL27*syn*3 locus). At least two glycoproteins possess penetration domains (gD and gH), from which gH initiates the penetration process. The gD penetration domain was localized to the N-terminus

Table III

Envelope glycoproteins in herpes simplex virus replication*

Glyco-protein	Gene	Function
gB	UL27	Interacts with GAGs during early adsorption, participates in membrane fusion (syn3 mutations are clustered to the C-terminal endodomain regions I and II). Its ectodomain contains many neutralizing epitopes.
gC	UL44	Cooperates with other glycoproteins during adsorption. Interacts with GAGs and complement (C3). Contains at least 2 discontinuous domains which can be modified by O-glycosylation. Reacts with lectins *(Helix pomatia)*. HSV-1 or HSV-2 specific antigenic domain.
gD	US6	Interacts with protein cell receptors (HveC/nectin-1, member of the immunoglobulin superfamily and HveA, member of the TNF receptor family), has a fusion domain and contains several discontinuous antigenic epitopes to which neutralizing antibodies bind. Participates in cell-to-cell spread and neural (transsynaptic) spread.**
gE	US8	Reacts with the Fc receptor, mediates cell-to-cell transmission and virion adsorption to nerve endings (gE/gI complex). Not essential for replication *in vitro,* but important *in vivo*. Complexes with gI.
gG	US4	Specific sequences present in HSV-2, some regions in HSV-1 deleted. Unknown but clear-cut role in virion entry.
gH	UL22	Forms complex with gL. Initiates membrane fusion (a fusion initiation domain about 100 aa long located just before the transmembrane domain and a fusion-associated SVP motif at aa 830–832) and stabilizes late adsorption. Contains several neutralizing epitopes.
gI	US7	Complexes with gE.
gK	UL53	Present in nuclear membrane in lower amounts in the cell mambrane, nearly absent in virions. Involved in virion transport across cytoplasm and in the envelopment of mature capsids. Contains syn1 mutations.
gL	UL1	Forms a complex with gH, some epitopes are present in the gH/gL complex only.

* according to Rajčáni and Vojvodová [94]

** Rajčáni et al. [143]

Table IV

Non-structural and structural non-evelope proteins related to HSV virulence

Protein	Gene	Possible role for virulence	
ICP0 (110 kDa)	RL2*	Non-structural immediate early (IE) protein	Universal cofaktor of HSV DNA transcription. Needed for efficient replication of HSV at the portal of entry.
ICP4 (175 kDa)	RS1**	Non-structural IE (α) protein	Transactivates the β- and γ-gene expression, down-regulates the α-genes. Essential for HSV DNA transciption at any replication site and during reactivation of latency.
ICP22 (68 kDa)	US1	Non-structural IE (α) protein	Activates β- and γ-gene transcription and regulates mRNA translation. Deletion mutant fails to express γ (capsid) proteins efficiently [144].
ICP27 (63 kDA)	UL54	Non-structural IE (α) protein	Inhibits splicing of cellular mRNA; promotes viral mRNA transport and polyadenylation.
ICP6	UL39	Non-structural early (β) protein	Large subunit for ribonucleotide reductase (RR1). Needed for efficient virus growth at the portal of entry [145–147].
Thymidinkinase	UL23	Non-structural early (β) protein	Essential for virus replication in neurons and for the colonization of regional sensory ganglion. Important helper protein for establishing latency at reactivation [92, 148].
DNA polymerase	UL30	Non-structural early (β) protein	DNA polymerase and related proteins for DNA synthesis. Important for productive HS replication at portal of entry as well as in neurons; pivotal protein following reactivation of latency [149].
α-TIF, VP16 M_r 65	UL48	Structural tegument protein	Activates (initiates) α-protein transcription (transscription initiation factor). Essential for HSV replication to high titers at portal of entry and for productive replication in neurons (in its absence latency is easily established).
(ICP34.5) γ34.5	RL1	Structural tegument protein	Neurovirulence factor for HSV replication within CNS [150]. The C-terminus contains a peptide region homologous to the GADD34 death domain and to Myd116 protein in leukemia cells, required to preclude total shutoff of protein synthesis at DNA damage [112].

* localized in the repeat flanking the UL segment
** localized in the repeat flanking the US segment

of the polypeptide chain, more precisely to the vicinity of residues 25 and 47 [93]. At progressed adsorption, the gD and probably also gB provide stable attachment to cellular protein receptors, in general termed herpes virus entry mediators (HVEM) (reviewed in [94]). Irreversible adsorption and initiation of penetration occurs especially after interaction of gD with the cellular adhesion molecule

termed HveC/nectin1 [95]. A further HSV glycoprotein heterodimer termed gE/gI has been implicated in cell-to-cell spread (interaction with the cell junctions of squamous epithelium cells) by avoiding virion release from infected cells [96]. Furthermore, gE interacts with nerve endings and possibly represents an important virulence factor for HSV attachment to the free nerve endings in the skin and mucous membranes, following even minimal virus replication at the portal of entry. The gEdel HSV-1 would not spread along nerves as demonstrated in mice after virus inoculation into lip and cornea [97]. Finally, gK plays essential role in capsid envelopment at nuclear membrane and in virion transport to cell surface [98].

The genetic background of HSZP virus, an HSV-1 strain with extensive passage history, was studied by parallel comparative sequencing of 4 relevant genes (UL27/gB, UL41/vhs, UL44/gC and UL53/gK) of HSZP and of additional selected viruses (strains ANGpath, strain KOS and the prototype strain 17). Mutation at aa 858 (*His* for Arg) in gB of the HSZP was found responsible for giant cell formation (syn^3gB mutation), similarly as the aa 855 mutation (*Val* for Ala) in gB of ANGpath [99, 100]. No syn^1gK mutations were detected in the UL53 gene either of HSZP or of ANGpath viruses [101]. The reduced virulence of HSZP for adult mice after peripheral inoculation, similarly as that of KOS virus, seems to be related to numerous mutations in the gB ectodomain. From these two mutations located in the antigenic domain IV were the same in gB^{HSZP} as well as in gB^{KOS} (at aa 59 and 79), at least one or two (aa 553; aa 515) were specific for gB^{KOS}, and one mutation (*Ser* for Ala at aa 108) was specific for gB^{HSZP} [102]. The latter gB^{HSZP} mutation and the above-mentioned gB^{KOS} mutations might have been related to the decreased virulence of HSZP and/or KOS. The assumption that gB might have some role for HSV pathogenicity comes from the replacement of gB ANGpath for gB KOS in the ANGpath DNA [103]. It had been suggested that higher virulence of ANGpath [104] might be associated with the syn^3 marker [105], but this could not be confirmed since the non-pathogenic HSZP strain has a similar syn^3 mutation [106]. Summing up, mutations in the variable region of gB^{HSZP} and gB^{KOS} between aa 59–108 are responsible for decreased pathogenicity [107], while the virulence of gBKOS is further abolished by the presence of mutations in the so-called entry domain [108] at residues 553 and 515, respectively [102, 109]. The UL44 gene coding for gC^{HSZP} had two specific mutations (*Trp* at aa 137 and 147 for Arg) located in antigenic domain II of gC, within the epitope which is responsible for binding of HSV-1 virions to the glycosoaminoglycan (GAG) receptor [110]. However, these mutations are irrelevant for virion adsorption due to the presence of an alternative GAG binding domain in gB. In addition to the envelope glycoproteins (Table III) which are either essential (gB, gD and gH) or non-essential (gC and gE)

for replication *in vitro*, several tegument proteins and some non-structural early as well as immediate early proteins are important tools of HSV virulence (Table IV). Experiments with several other deletion mutants, such as the HFEM strain deleted in the latency associated transcript coding sequence (LAT), in UL56 and UL55 genes showed that the DNA region at MU 0.762–0.789 may also contribute to virulence [111]. Finally, neurovirulence of the HSV strains was associated with the γ34.5 protein encoded by repetitive region flanking the UL segment [112].

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References

1. Murphy, B. R., Hinshaw, V. S., Sly, D. L.: Virulence of avian influenza A viruses for squirrel monkeys. Infect Immun **37,** 1119–1126 (1982).
2. Stott, E. J., Killington, R. A.: Rhinoviruses. Ann Rev Microbiol **26,** 503–524 (1972).
3. Melnick, L. J.: Enteroviruses: Polioviruses, Coxsackieviruses, Echoviruses and newer Enteroviruses. In: Fields, B. N., Knipe, D. M., Howley, P. M. (eds): Fields Virology, 3rd ed., Lipincott Raven, 1996, pp. 655–712.
4. Craighead, J. E.: Enteroviruses. In: Pathology and pathogenesis of human viral disease, Academic Press, San Diego, 2000, pp. 1–28.
5. Bodian, D.: Poliomyelitis. In: Rivers, T. M., Horsfall, F. L. (eds): Viral and Rickettsial Infections of Man, 3rd ed., J. B. Lippincott, Philadelphia, 1959, pp. 430–473.
6. Rueckert, R. R.: Picornaviridae: the viruses and their replication. In: Fields, B. N., Knipe, D. M., Howley, P. M. (eds): Fields Virology, 3rd ed., Lipincott Raven, 1996, pp. 609–654.
7. Horie, H., Koike, S., Kurata, T., Sato-Yoshida, T., Ise, I., Ota, Y., Abe, S., Hioki, K., Kato, H., Taya, C., Nomura, T., Hashizume, S., Yonekawa, H., Nomoto, A.: Transgenic mice carrying the human poliovirus receptor: new animal model for study of poliovirus neurovirulence. J Virol **68,** 681–688 (1994).
8. McGoldrick, A., Macadam, A. J., Dunn, G., Rowe, A., Burlison, J., Minor, P. D., Meredith, J., Evans, D. D. J., Almond, J. W.: Role of mutations G-480 and C-6203 in the attenuation phenotype of Sabin poliovirus. J Virol **69,** 7601–7605 (1995).
9. Rezapkin, G. V., Norwood, L. P., Taffs, R. E., Dragunsky, E. M., Levenbook, I. S., Chumakov, K. M.: Microevolution of type 3 Sabin strain of poliovirus un cell cultures and its implications for oral poliovirus vaccine quality control. Virology **211,** 377–378 (1994).
10. Le, S. Y., Zucker, M.: Common structures of the 5'-non-coding RNA in enteroviruses and rhinoviruses: thermodynamical stability and statistical significance. J Mol Biol **216,** 729–741 (1990).
11. Friedrich, F.: Genomic modifications in Sabin vaccine strains isolated from vaccination-associated cases, healthy contacts and healthy vaccinees. Acta Virol **40,** 157–170 (1996).
12. Friedrich, F.: Neurologic complications associated with oral poliovirus vaccine and genomic variability of the vaccine strains after multiplication in humans. Acta Virol **42,** 187–195 (1998).

13. Hogle, J. M., Chow, M., Filman, D.: Three dimensional structure of poliovirus at 2.9 A resolution. Science **229,** 1358–1365 (1985).
14. Colonno, R. J.: Cell surface receptors for picornaviruses. BioEssays **5,** 270–274 (1986).
15. Huber, A. A.: VCAM-1 is a receptor for encephalomyocarditis virus on murine vascular endothelial cells. J Virol **68,** 3453–3458 (1994).
16. Bergelson, J. M., Shepley, M. P., Chan, B. M. C.: Identification of the integrin VLA-2 as a receptor for Echovirus. Science **255,** 1718–1720 (1992).
17. Mendelsohn, C., Wimmer, E., Racaniello, V.: Cellular receptor for poliovirus: molecular cloning, nucleotide sequence and expression of a new member of the immunoglobulin super-family. Cell **56,** 855–865 (1989).
18. Badger, J., Minor, I., Dremer, M. J., Oliveira, M. A., Smith, T. J., Griffith, J. P.: Structural analysis of a series of antiviral agents complexed with human rhinovirus 14. Proc Natn Acad Sci USA **85,** 3304–3308 (1988).
19. Fox, G., Parry, N. R., Barnett, P. V., McGinn, B., Rowlands, D. J., Brown, F.: The cell attachment site on foot-and-mouth disease virus includes the amino acid sequence RDG (arginine-glycine-aspartic acid). J Gen Virol **70,** 625–637 (1989).
20. Madshus, I. H., Olsnes, S., Sandwig, K.: Mechanism of entry into the cytosol of polivirus type 1: requirement of low pH. J Cell Biol **98,** 1194–1200 (1984).
21. Krug, R. M., Alonso-Caplen, F. V., Julkunen, I., Katz, M. G.: Expression and replication of the influenza virus genome. In: Krug, R. M. (ed.): The influenza viruses, Plenum Press, New York, 1989, pp. 89–152.
22. Lamb, R. A., Krug, R. M.: Orthomyxoviridae: the viruses and their replication. In: Fields, B. N., Knipe, D, M., Howley, P. M. (eds): Fields Virology, 3rd ed., Lipincott Raven, 1996, pp. 1353–1395.
23. Li, M. L., Ramirez, B. C., Krug, R. M.: RNA-dependent activation of primer RNA production by influenza virus polymerase: different regions of the same protein subunit constitute the required RNA binding sites. EMBO J **17,** 5844–5852 (1998).
24. Krug, R. M.: Priming of influenza viral RNA transcription by capped heterologous RNAs. Curr Top Microbiol Immunol **93,** 125–149 (1981).
25. Plotch, S. J., Bouloy, M., Ulmanen, I., Krug, R. M.: A unique cap (m7GpppNXm)-dependent endonuclease cleaves capped RNAs to generate the primers that induce viral RNA transcription. Cell **23,** 847–858 (1981).
26. Beaton, A. R., Krug, R. M.: Transcription antitermination during influenza virus template RNA synthesis requires nucleocapsid protein and the absence of a 5'-capped end. Proc Natn Acad Sci USA **113,** 165–197 (1977).
27. Shapiro, G. I., Krug, R. M.: Influenza virus RNA replication in vitro: synthesis of viral template RNAs amd virion RNAs in the absence of an added primer. J Virol **62,** 2285–2290 (1988).
28. Chen, Z., Li, Y., Krug, R. M.: Inluenza virus protein targets poly(A)-binding protein II of the cellular 3' -end processing machinery. EMBO J **18,** 2273–2283 (1999).
29. Verhoeyen, M., Fang, R., Min Jou, W., Devos, R., Huylebroeck, D., Saman, E., Fiers, W.: Antigenic drift between the hemagglutinin of the Hong-Kong influenza strains A/Aichi/2/68 and A/Vicoria/3/75. Nature **286,** 771–776 (1980).
30. Lamb, R. A., Chopin, P. W.: The gene structure and replication of influenza virus. Ann Rev Biochem **52,** 467–506 (1983).

31. Nobusawa, E., Aoyama, T., Kato, H., Suzuki, Y., Tateno, Y., Nakajima, K.: Comparison of complete amino acid sequences and receptor binding properties among 13 serotypes of hemagglutinin of influenza A viruses. Virology **182,** 475–485 (1991).
32. Wilson, I. A., Skehel, J. J., Wiley, D. C.: Structure of the hemagglutinin membrane glycoprotein of influenza virus at a 3 A resolution. Nature **289,** 366–373 (1981).
33. Doms, R. W., Lamb, R. A., Rose, J. K., Helenius, A.: Folding and assembly of viral membrane proteins. Virology **193,** 545–562 (1993).
34. Garten, W., Bosch, F. X., Linder, D., Rott, R., Klenk, H. D.: Proteolytic activation of the influenza virus hemagglutinin: the structure of the cleavage site and the enzymes involved in cleavage. Adv Exp Med Biol **142,** 119–131 (1981).
35. Ogasawa, T., Botoh, B., Suzuki, H., Asaka, J., Shimokata, K., Rott, R., Nagaui, Y.: Expression of factor X and its significance for the determination of paramyxovirus tropism in the chick embryo. EMBO J **11,** 467–472 (1992).
36. Klenk, H. D., Garten, W.: Host cell proteases controlling virus pathogenicity. Trends in Microbiol **2,** 39–43 (1994).
37. Murphy, B. R., Webster, R. G.: Orthomyxoviruses. In: Fields, B. N., Knipe, D, M. (eds): Fields Virology, 2nd ed., Raven Press Ltd., New York, 1990, pp. 1091–1152.
38. Wharton, S. A., Martin, S. R., Ruigrok, R. W. H., Kehel, J. J., Wiley, D. C.: Membrane fusion by peptide analogues of influenza virus hemagglutinin. J Gen Virol **69,** 1847–1857 (1988).
39. Skehel, J. J., Bayley, P. M., Brown, E. B.: Changes in the conformation of influenza virus hemagglutinin at the pH optimum of virus-mediated membrane fusion. Proc Natn Acad Sci USA **53,** 968–972 (1982).
40. Stegman, T., Booy, F. P., Witschut, J.: Effects of low pH on influenza virus: activation and inactivation of the membrane fusion capacity of the hemagglutinin. J Biol Chem **262,** 17744–17749 (1987).
41. Wilson, I. A., Skehel, J. J., Wiley, D. C.: Structure of the hemagglutinin membrane glycoprotein of influenza virus at 3 A resolution. Nature **289,** 366–373 (1981).
42. Bullough, P. A., Hughson, F. M., Skehel, J. J., Wiley, D. C.: Structure of infleunza hemagglutinin at the pH of membrane fusion. Nature **371,** 37–43 (1994).
43. Wiley, D. C., Skehel, J. J.: The structure and function of the hemagglutinin membrane glycoprotein of influenza virus. Ann Rev Biochem **56,** 365–394 (1987).
44. Weis, W., Brown, I. M., Cusack, S.: Structure of the influenza virus haemagglutinin complexed with its receptor sialic acid. Nature **333,** 426–431 (1988).
45. Rogers, G. N., Paulson, J. C., Daniels, R. S., Skehel, J. J., Wilson, I. A., Wiley, D. C.: Single amino acid substitution in influenza hemagglutinin change receptor binding specificity. Nature **304,** 76–78 (1983).
46. Scholtissek, C., Rohde, W., Harms, E., Rott, R.: Assignments of gene functions influenza A2-Singapore to genetic relatedness to other influenza strains. In: Barry, R. D., Mahy, B. W. J. (eds): Negative strand viruses and the host cell, Academic Press, London, 1978, pp. 19–25.
47. Fang, R., Min Jou, W., Huylebroeck, D., Devos, R., Fiers, W.: Complex structure of A/Duck/Ukraine/63 influenza hemagglutinatin gene: animal virus progenitor of human H3 Hong Kong 1968 influenza hemagglutinin. Cell **25,** 237–307 (1979).
48. Murphy, B. R., Webster, R. G.: Orthomyxoviruses. In: Fields, B. N., Knipe, D. M., Howley, P. M. (eds): Fields Virology, 3rd ed., Lipincott Raven, 1996, pp. 1397–1445.

49. Sugrue, R. J., Hay, A. J.: Structural characteristics of the M2 protein of influenza A viruses: evidence that it forms a tetrameric channel. Virology **179,** 617–624 (1991).
50. Oxford, J. S., Galbraith, A.: Antiviral activity of amantadine: a review of laboratory and clinical data. Pharmacol Ther **11,** 181–262 (1980).
51. Hay, A. J.: The action of amantanamines against influenza viruses: inhibition of the M2 ion channel protein. Semin Virol **3,** 21–30 (1992).
52. Dietschold, B., Rupprecht, Ch. E., Zhen Fang, Fu., Koprowski, H.: Rhabdoviruses. In: Fields, B. N., Knipe, D. M., Howley, P. M. (eds): Fields Virology, 3rd ed., Lipincott Raven, 1996, pp. 1137–1159.
53. Anilionis, A., Wunner, W. H., Curtis, P.: Structure of the glycoprotein gene of rabies virus. Nature (London) **294,** 275–278 (1985).
54. Harrison, A. K., Murphy, F. A.: Lyssavirus infection of muscle spindles and motor end plates in striated muscle of hamsters. J Arch Virol **57,** 167 (1978).
55. Watson, H. D., Tignor, G. H., Smith, A. L.: Entry of rabies virus into the peripheral nerves of mice. J Gen Virol **56,** 371–382 (1981).
56. Lentz, T. L., Burrage, T. G., Smith, A. L., Crick, J., Tignor, G.: Is the acetylcholine receptor a rabies virus receptor? Science **215,** 182–184 (1982).
57. Burrage, T. G., Tignor, G. H., Smith, A. L.: Rabies virus binding at neuromuscular junctions. Virus Res **2,** 273–289 (1985).
58. Lentz, T. L., Benson, R. J. J., Klimovicz, D., Wilson, P. T., Hawrot, E.: Binding of rabies virus to purified Torpedo acetylcholine receptor. Mol Brain Res **1,** 211–219 (1986).
59. Dietschold, B., Wiktor, T. J., Trojanovski, J. Q.: Differences in cell to cell spread of pathogenic and apathogenic rabies virus strains in vivo and in vitro. J Virol **56,** 12–18 (1985).
60. Coulon, P., Rollin, P. E., Flamand, A.: Molecular basis of rabies virus virulence. II. Identification of a site on the CVS glycoprotein associated with virulence. J Gen Virol **64,** 693–696 (1983).
61. Whitt, M. A., Zagouras, P., Crise, B., Rose, J. K.: A fusion defective mutant of the vesicular stomatitis virus glycoprotein. J Virol **64,** 4907–4913 (1990).
62. Morimoto, K., Ni, Y. J., Kawai, A.: Syncytium formation is induced in the murine neuroblastoma cell cultures which produce pathogenic type G proteins of the rabies virus. Virology **189,** 203–216 (1992).
63. Tsiang, H.: Evidence for an intraaxonal transport of fixed and street rabies virus. J Neuropathol Exp Neurol **38,** 286–292 (1978).
64. Tsiang, H.: An *in vitro* study of rabies pathogenesis. Bull Inst Pasteur **83,** 41–56 (1985).
65. Nibert, M. L., Schiff, L. A., Fields, B. N.: Reoviruses and their replication. In: Fields, B. N., Knipe, D. M., Howley, P. M. (eds): Fields Virology, 3rd ed., Lipincott Raven, 1996, pp. 1557–1596.
66. Weiner, H. L., Drayna, D., Averill, D. R.: Molecular basis of reovirus virulence: role of S1 gene. Proc Natn Acad Sci USA **74,** 5744–5748 (1977).
67. Lee, P. W. K., Hayes, E. C., Joklik, W. K.: Protein σ1 is the reovirus cell attachment protein. Virology **108,** 156–163 (1981).
68. Masri, S. A., Nagata, L., Mah, D. C., Lee, P. W.: Functional expression in *E. coli* of cloned reovirus σ1 gene encoding the viral cell attachment target protein σ1. Virology **149,** 83–90 (1986).
69. Gentsch, J., Pacitti, A.: Differential interaction of reovirus type 3 with sialylated components on animal cells. Virology **161,** 245–248 (1987).

70. Paul, R. W., Choi, A. H., Lee, P. W. K.: The alpha-anomeric form of sialic acid is the minimal receptor determinant recognized by reovirus. Virology **172,** 382–385 (1989).
71. Tang, D., Strong, J. E., Lee, P. W. K.: Recognition of the epidermal growth factor receptor by reovirus. Virology **197,** 412–414 (1993).
72. Choi, A. H. C., Lee, P. W. K.: Does the beta-adrenergic receptor function as a reovirus receptor? Virology **163,** 191–197 (1988).
73. Choi, A. H. C., Paul, R. W., Lee, P. W. K.: Reovirus binds to multiple plasma membrane proteins of mouse L fibroblasts. Virology **178,** 316–320 (1990).
74. Sauve, G. J., Saragovi, H. U., Greene, M. I.: Reovirus receptors. Adv Virus Res **42,** 232–341 (1992).
75. Dermody, T. S., Nibert, M. L., Bassel-Duby, R., Fields, B. N.: A S1 region important for hemagglutination by serotype 3 reovirus strains. J Virol **64,** 5173–5176 (1990).
76. Huismans, H., Joklik, W. K.: Reovirus coded polypeptides in infected cells: isolation of two native monomeric polypeptides with high affinity for single stranded and double stranded RNA, respectively. Virology **70,** 411–424 (1976).
77. Lemioux, R., Lemay, G., Millward, S.: The viral protein σ3 participates in translation of late viral messanger RNA in reovirus infected cells. J Virol **61,** 2472–2479 (1987).
78. Schiff, L. A., Nibert, M. L., Co, M. S., Brown, E. G., Fields, B. N.: Distinct binding sites for zinc and double stranded RNA in the reovirus outer capsid protein σ3. Mol Cell Biol **8,** 273–282 (1984).
79. Lloyd, R. M., Shatkin, A. J.: Translational stimulation by reovirus polypeptide σ3: substitution for VA1-RNA and inhibition of phosphorylation of the a-subunit of eukaryotic initiation factor-II. J Virol **66,** 6878–6884 (1992).
80. Dryden, K. A., Wang, G., Yeager, M., Nibert, M. L., Coombs, K. M., Furlong, D. B., Field, B. N., Baker, T. S.: Early steps in reovirus infection are associated with dramatic changes in supramiolecular structure and protein conformation: analysis of virions and subviral particles by cryoelectron microscopy and image reconstruction. J Cell Biol **122,** 1023–1041 (1993).
81. Bodkin, D. K., Nibert, M. L., Fields, B. N.: Proteolytic digestion of reovirus in the intestinal lumens of neonatal mice. J Virol **63,** 4676–4681 (1989).
82. Bass, D. M., Bodkin, D., Dambrauskas, R., Trier, J. S., Fields, B. N., Wolf, J. L.: Intraluminal proteolytic activation plays an importany role in replication of type 1 reovirus in the intestines of neonatal mice. J Virol **64,** 1830–1833 (1990).
83. Tillotson, L., Shatkin, A. J.: Reovirus polypeptide σ3 and N-terminal myristoylation of polypeptide μ1/μ1C is required for site specific cleavage to μ1C in transfected cells. J Virol **66,** 2180–2186 (1992).
84. Nibert, M. L., Fields, B. N.: A carboxy terminal fragment of protein μ1/μ1C is present in infectious subvirion particles of mammalian reoviruses and is proposed to have a role in penetration. J Virol **66,** 6408–6418 (1992).
85. Sturzenbecker, L. J., Nibert, M., Furlong, D., Fields, B. N.: Intracellular digestion of reovirus particles requires a low pH and is an essential step in the viral infectious cycle. J Virol **61,** 2351–2361 (1987).
86. Armstrong, G. D., Paul, R. W., Lee, P. W.: Studies on reovirus receptors of L-cells: virus binding characteristics and comparison with reovirus receptors of erythrocytes. Virology **138,** 37–48.
87. Estes, M. K.: Rotaviruses and their replication. In: Fields, B. N., Knipe, D. M., Howley, P.M. (eds): Fields Virology, 3 rd ed., Lipincott Raven, 1996, pp. 1625–1655.

88. Tyler, K. L., Fields, B. N.: Reoviruses. In: Fields, B. N., Knipe, D. M., Howley, P. M. (eds): Fields Virology, 3rd ed., Lipincott Raven, 1996, pp. 1597–1623.
89. Tyler, K. L., Fields, B. N.: Pathogenesis of viral infections. In: Fields, B. N., Knipe, D. M., Howley, P. M. (eds): Fields Virology, 3rd ed., Lipincott Raven, 1996, pp. 173–217.
90. Whitley, R. J.: Herpes simplex viruses. In: Fields, B. N., Knipe, D. M., Howley, P. M. (eds): Fields Virology, 3rd ed., Lipincott Raven, 1996, pp. 2297–2342.
91. Roizman, B., Sears, A. E.: Herpes simplex viruses and their replication. In: Fields, B. N., Knipe, D. M., Howley, P. M. (eds): Fields Virology, 3rd ed., Lipincott Raven, 1996, pp. 2231–2295.
92. Rajčáni, J., Ďurmanová, V.: Early expression of herpes simplex virus proteins and reactivation of latent infection. Folia Microbiol **45,** 7–28 (2000).
93. Rajčáni, J., Vojvodová, A: The role of herpes simplex virus glycoproteins in the virus replication cycle. Acta Virol. **42,** 103–118 (1998).
94. Krumenacher, C., Baribaud, I., Kwan, L., Spear, P., Eisenberg, R., Cohen, G.: Effects of glycoprotein D binding and HSV infection on HveC/nectin 1 activity as a cell adhesion molecule. 2.02, 26th International Herpesvirus Workshop, 2001.
95. Lingen, M., Seck, T., Dehoust, U., Weise, K., Falke, D.: Amino acid substitutions in gD mediate the ability of "fusion from without" – positive herpes simplex type 1 strains to penetrate at 4 ºC in the presence of soluble glycoprotein D. Intervirology **38,** 283–289 (1994).
96. Dingwell, K. S., Brunetti, C. R., Hendricks, R. L., Tang, Q., Tang, M., Rainbow, A. J., Johnson, D. C.: Herpes simplex virus glycoprotein E and I facilitate cell to cell spread in vivo and across junctions of cultured cells. J Virol **68,** 834–845 (1994).
97. Rajčáni, J., Herget, U., Kaerner, H. C.: Spread of herpes simplex virus (HSV) strains SC16, ANG, ANGpath and its gC minus and gE minus mutants in DBA-2 mice. Acta Virol 305–320 (1990).
98. Rajčáni, J., Kúdelová, M.: Glycoprotein K of herpes simplex virus: a transmembrane protein encoded by the UL53 gene which regulates membrane fusion. Virus Genes **18,** 81–90 (1998).
99. Gage, P. J., Levine, M., Glorioso, J. C.: Syncytium-inducing mutations localize to two discrete regions within the cytoplasmic domain of herpes simplex virus type 1 glycoprotein B. J Virol **67,** 2191–2201 (1993).
100. Rajčáni, J., Kúdelová, M., Oravcová, I., Vojvodová, A., Košovsdký, J., Matis, J.: Characterization of strain HSZP of herpes simplex virus type 1. Folia Microbiol **44,** 713–719 (1999).
101. Kúdelová, M., Vojvodová, A., Rajčáni, J.: Syn strains HSZP and ANGpath of herpes simplex virus type 1 do not contain mutations in the regions of UL53 gene relevant to syncytium formation. Acta Virol **42,** 41–45 (1998).
102. Košovský, J., Vojvodová, A., Oravcová, I., Kúdelová, M., Matis, J., Rajčáni, J.: Herpes simplex virus strain HSZP glycoprotein B gene: comparison of mutations among strains differing in virulence. Virus Genes **20,** 25–31 (2000).
103. Košt'ál, M., Bačík, I., Rajčáni, J., Kaerner, H.: Replacement of glycoprotein B gene in the herpes simplex type 1 strain ANGpath DNA by that originating from nonpathogenic strain KOS reduces the pathogenicity of recombinant virus. Acta Virol **38,** 77–88 (1994).
104. Kumel, G., Schroder, C. H., Kaerner, H. C.: Neurovirulence and latency in inbred mice: two HSV-1 intrastrain variants of different pathogenicity. Med Microbiol Immunol **174,** 313–324 (1986).

105. Weise, K., Kaernaer, H. C., Glorioso, J., Schroder, C. H.: Replacement of glycoprotein B gene sequences in herpes simplex virus type 1 strain ANG by corresponding sequences of the strain KOS causes changes in plaque morphology and neuropathogenicity. J Gen Virol **68,** 1909–1919 (1987).
106. Rajčáni, J., Vojvodová, A., Matis, J., Kúdelová, M., Dragúňová, J., Krivjanská, M., Zelník, V.: The syn3 strain HSZP of herpes simplex virus type 1 is not pathogenic for mice and shows limited neural spread. Virus Res **43,** 33–44 (1996).
107. Yuhasz, S., Stevens, G.: Glycoprotein B is a specific determinant of herpes simplex type 1 neuroinvasiveness. J Virol **67,** 5948–5954 (1993).
108. DeLuca, N. A., Bzik, D., Bond, V. C., Person, S., Snipes, W.: Nucleotide sequences of herpes simplex virus type 1 affecting virus entry, cell fusion and production of gB (VP7). Virology **122,** 411–423 (1982).
109. Sakharkiz-Langroody, A., Holland, T.: Identification of fusion-from-without determinants of herpes simplex virus glycoprotein B. Virology **227,** 153–159 (1997).
110. Oravcová, I., Kúdelová, M., Mlčuchová, J., Matis, J., Bystrická, M., Westra, D. F., Wellin-Wester, G. W., Rajčáni, J.: Characterization of glycoprotein C from the strain HSZP of herpes simplex 1. Acta Virol **44,** 99–108 (2000).
111. Rosen, A., Ernst, F., Koch, H. G., Gederblom, H., Darai, G., Hadar, J., Tabor, E., Ben-Hur, T., Becker, Y.: Replacement of the deletion in the genome (0.762–0.789) of virulent HSV-1 HFEM using cloned MluI DNA fragment (0.7615–0.796) of virulent HSV-1 F leads to generation of virulent intratype recombinant. Virus Res **5,** 157–175 (1986).
112. Chou, J., Roizman, B.: The γ34.5 gene of herpes simplex virus function which blocks the host response to infection maps in the homologous domain of the genes expressed during growth arrest and DNA damage. Proc Natn Acad Sci USA **91,** 5247–5251 (1994).
113. Wickham, T. J., Mathias, P., Cheresh, D. A., Nemerow, G. W.: Integrins $a_v\beta_3$ and $a_v\beta_5$ promote adenovirus internalization but not virus attachment. Cell **73,** 309–319 (1993).
114. Yokomori, K., Lai, M. C.: Mouse hepatitis virus utilizes two carcinoembryonic antigens as alternative receptors. J Virol **66,** 6194–6199 (1992).
115. Dveskler, G. S., Dieffenbach, C. W., Cardellichio, C. B.: Several members of the carcinoembryonic antigen-related glycoprotein family are functional receptors for the coronavirus mouse hepatitis virus A59. J Virol **67,** 1–7 (1993).
116. Yeager, C. L., Ashman, R. A., Wiliams, R. K.: Human aminopeptidase N is a receptor for coronavirus 229E. Nature **357,** 420–422 (1992).
117. Nemerow, G. R., Wolfert, R., McNaughton, M. E.: Identification and characterization of the Epstein–Barr virus receptor on human B lymphocytes and its relationship to the C3d complement receptor (CR2). J Virol **55,** 345–351 (1985).
118. Fingeroth, J. D., Clabby, M. L., Strominger, J. D.: Characterization of a T-lymphocyte Epstein–Barr virus/C3d receptor (CD21). J Virol **62,** 1442–1447 (1988).
119. McClure, J. E.: Cellular receptor for Epstein–Barr virus. Progr Med Virol **39,** 116–138 (1992).
120. Shich, M. T., Wudunn, D., Montgomery, R. I.: Cell surface receptors for herpes simplex virus are heparine sulphate proteoglycans. J Cell Biol **116,** 1273–1281 (1992).
121. Spear, P. G.: Entry of alpha-herpesviruses into cells. Semin Virol **4,** 167–180 (1993).
122. Campadelli-Fiume, G., Cocchi, F., Menotti, L., Lopez, M.: The novel receptors that mediate entry of herpes simplex viruses into cells. Rev Med Virol **10,** 395–319 (2000).
123. Grundy, J. E., McKeating, J. A., Griffits, P. D.: Cytomegalovirus strain AD169 binds beta-2 microglobulin *in vitro* after release from cells. J Gen Virol **68,** 777–784 (1987).

124. Compton, T., Nowlin, D. M., Cooper, N. R.: Initiation of human cytomegalovirus infection requires initial interaction with cell surface heparan sulfate. Virology **193,** 834–841 (1993).
125. Wykes, M. N., Shellam, G. R., McCluskey, J.: Murine cytomegalovirus interacts with major histocompatibility complex I molecules to establish cellular infection. J Virol **67,** 4182–4189 (1993).
126. Higa, H. H., Rogers, G. N., Paulson, J. C.: Influenza virus hemagglutinins differentiate between receptor determinants bearing N-acetyl-N-glycollyl- and N-O-diacetylmuraminic acid groups. Virology **144,** 279–282 (1985).
127. Weiss, R. A.: Human immunodeficiency virus receptors. Semin Virol **3,** 426–431 (1992).
128. Greve, J. M., Davies, G., Meyer, A. M.: The major rhinovirus receptor is ICAM-1. Cell **56,** 839–847 (1989).
129. Staunton, D. E., Merluzzi, V. J., Rothlein, R.: A cell adhesion molecule, ICAM-1, is the major surface receptor for rhinoviruses. Cell **56,** 849–853 (1989).
130. Bergelson, J. M., St. John, N., Kawaguchi, S.: Infection by Echoviruses 1 and 8 depends on the alpha-2 subunit of human VLA-2. J Virol **67,** 6847–6852 (1993).
131. Epstein, D. A., Marsh, Y. V., Schreiber, A. B.: Epidermal growth factor receptor occupancy inhibits vaccinia virus infection. Nature **318,** 663–665 (1985).
132. Dalgleish, A. G., Beverly, P. C. L., Clapham, P. R.: The CD4 antigen is an essential component of the receptor for the AIDS retrovirus. Nature **312,** 763–767 (1985).
133. Klatzman, D., Champagne, E., Charamaret, S.: T-lymphocyte T4 molecule behaves as the receptor for human retrovirus LAV. Nature **312,** 767–768 (1984).
134. Sattentau, Q. J., Weiss, R. A.: The CD4 antigen: physiologic ligand and HIV receptor. Cell **52,** 631–633 (1988).
135. Weiss, R. A.: Human immunodeficiency virus receptors. Semin Virol **3,** 79–84 (1992).
136. Bhat, S., Spitalnik, S. L., Gonzalez-Scarano, F.: Galactosyl ceramide or a derivative is an essential component of the neural receptor for human immunodeficiency virus type 1 envelope glycoprotein gp120. Proc Natn Acad Sci USA **88,** 7131–7134 (1991).
137. Lentz, T.: Rabies virus receptors. Trends Neurosci **8,** 360–364 (1985).
138. Hahnham, C. A., Zhao, F., Tignor, G. H.: Evidence from the anti-idiotype network that the acetylcholine receptor is a rabies virus receptor. J Virol **67,** 530–542 (1993).
139. Superti, F., Hauttecocoeur, B., Morelec, M. J.: Involvement of gangliosides in rabies virus infection. J Gen Virol **67,** 47–56 (1986).
140. Schlegel, R., Tralka, S., Willingham, M. C.: Inhibition of VSV binding and infectivity of phosphatidylserine. Is phosphatidylserine a VSV binding site? Cell **32,** 639–646 (1983).
141. Wang, K. S., Kuhn, R. J., Strauss, E. G.: High affinity laminin receptor is a receptor for Sindbis virus in mammalian cells. J Virol **66,** 4992–5001 (1992).
142. Helenius, A., Morein, B., Fries, E.: Human HLA-A and HLA-B and murine H2K and H2D histocompatibility antigens are cell surface receptors for Semliki Forest virus. Proc Natn Acad Sci USA **75,** 3846–3850 (1978).
143. Rajčáni, J., Koštál, M., Kaerner, H. C.: Regions US6 and US7 of herpes simplex virus type 1 DNA encoding glycoproteins D and I may influence neuroinvasivity. Acta Virol **38,** 89–95 (1994).
144. Sears, A. E., Halliburton, I. W., Meignier, B., Silver, S., Roizman, B.: Herpes simplex virus 1 mutant deleted in the α22 gene: growth and gene expression in permissive and restrictive cells and establishment of latency in mice. J Virol **55,** 338–346 (1985).

145. Cameron, J. M., McDougall, I., Marsden, H. S., Preston, V. G., Ryan, D. M., Subak-Sharpe, J. H.: Ribonucleotide reductase encoded by herpes simplex virus is a determinant of the pathogenicity of the virus in mice and a valid antiviral target. J Gen Virol **69,** 2607–2612 (1988).
146. Jacobson, J. G.., Leib, D A., Doldstein, D. I., Bogard, C. C., Schaffer, Pam., Weller, S. K., Coen, D. M.: A herpes simplex virus ribonucleotide reductase deletion mutant is defective for productive, acute and reactivable infections of mice and for replication in mouse cells. Virology **3,** 276–283 (1989).
147. Brandt, C. R.., Kintner, R. L., Pumfrey, A. M., Visally, R. J., Grau, D. R.: The herpes simplex virus ribonucleotide reductase is required for ocular virulence. J Gen Virol **72,** 2043–2049 (1991).
148. Rajčáni, J.: DNA regions and genes determining virulence of herpes simplex virus. Acta Virol **36,** 208–222 (1992).
149. Preston, C. M.: Repression of viral transcription during herpes simplex virus latency. J Gen Virol **81,** 1–19 (2000).
150. Whitley, R. J., Kern, E., Chattopadhy, S., Chou, J., Roizman, B.: Replication establishment of latency, and induced reactivation of herpes simplex virus γ34.5 deletion mutants in rodent models. J Clin Invest **91,** 2837–2843 (1993).