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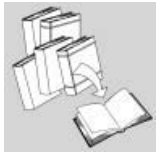
REVIEW



Transport and egress of herpes simplex virus in neurons

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SUMMARY

The mechanisms of axonal transport of the alphaherpesviruses, HSV and pseudorabies virus (PrV), in neuronal axons are of fundamental interest, particularly in comparison with other viruses, and offer potential sites for antiviral intervention or development of gene therapy vectors. These herpesviruses are transported rapidly along microtubules (MTs) in the retrograde direction from the axon terminus to the dorsal root ganglion and then anterogradely in the opposite direction. Retrograde transport follows fusion and deenvelopment of the viral capsid at the axonal membrane followed by loss of most of the tegument proteins and then binding of the capsid via one or more viral proteins (VPs) to the retrograde molecular motor dynein. The HSV capsid protein pUL35 has been shown to bind to the dynein light chain Tctex1 but is likely to be accompanied by additional dynein binding of an inner tegument protein. The mechanism of anterograde transport is much more controversial with different processes being claimed for PrV and HSV: separate transport of HSV capsid/tegument and glycoproteins versus PrV transport as an enveloped virion. The controversy has not been resolved despite application, in several laboratories, of confocal microscopy (CFM), real-time fluorescence with viruses dual labelled on capsid and glycoprotein, electron microscopy *in situ* and immuno-electron microscopy. Different processes for each virus seem counterintuitive although they are the most divergent in the alphaherpesvirus subfamily. Current hypotheses suggest that unenveloped HSV capsids complete assembly in the axonal growth cones and varicosities, whereas with PrV unenveloped capsids are only found travelling in a retrograde direction. Copyright © 2007 John Wiley & Sons, Ltd.

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Abbreviations used
APP, amyloid precursor protein; CFM, confocal microscopy; DRG, dorsal root ganglion; DYNC1H1, cytoplasmic dynein 1 heavy chain; DYNC1I, cytoplasmic dynein 1 intermediate chain; DYNC1LI, cytoplasmic dynein 1 light intermediate chain; DYNLL, cytoplasmic dynein light chain LC8; DYNLT1, cytoplasmic dynein light chain Tctex1; DYNLT3, cytoplasmic dynein light chain rp3; DYNLRB, cytoplasmic dynein light chain roadblock; GFP, green fluorescent protein; ICP, infected cell protein; KHC, kinesin heavy chain; KIF, kinesin superfamily; KLC, kinesin light chain; MT, microtubule; MTOC, microtubule-organising centre; mRFP, monomeric red fluorescent protein; PrV, pseudorabies virus; TEM, transmission electron microscopy; TIEM, transmission immunoelectron microscopy; TPR, tetratricopeptide repeat; U_L, unique long; U_S, unique short; VP, viral protein.

INTRODUCTION

The cycle of HSV infection

HSV type 1 (HSV-1) is one of the most common human pathogens, infecting 40–80% of people worldwide. It is a member of the alphaherpesvirus subfamily including VZV and pseudorabies virus (PrV). These herpesviruses are uniquely able to infect and remain latent within neurons. Although most clinical disease due to HSV-1 is relatively mild, it can sometimes cause encephalitis in adults, or disseminated infection in neonates, both of which are frequently fatal if untreated. Furthermore, mucocutaneous infection with HSV-1, especially in the genital region, causes significant morbidity, and reduced quality of life, for many people in the community. Genital herpes (HSV-1 or HSV-2) is a common problem but more so in the developing world where co-infection with

HIV leads to a two to four fold increase in HIV transmission [1].

One of the characteristic features of HSV-1 is its ability to infect neurons from where it is able to cause recurrent infections. After inoculation of the skin or mucous membrane, the majority of virus undergoes replication before entering dorsal root ganglion (DRG) sensory neurons. Here, it is transported from the nerve terminal along axons in a retrograde (backward step) direction to the neuronal cell body, where it establishes latent infection for the lifetime of the host. Periodic reactivation results in HSV-1 being transported in an anterograde (forward step) direction from the cell body to nerve axon terminals, where it causes either recurrent clinical disease, or asymptomatic viral shedding at the skin or mucous membranes of the same dermatome involved in the initial infection [2]. Because of the very long distances in neuronal axons, up to 1 m, that need to be traversed by HSV-1 (virion nuclear capsid diameter 100 nm), its transport must be an active process, and is thought to utilise cellular molecular motors, such as dynein and kinesin [3–7].

The alphaherpesviruses HSV and PrV have similar biology in cell lines but there is controversy about aspects of axonal transport and that is a central point of this review. Defining the mechanism of anterograde transport could facilitate development of new strategies for antiviral therapy for recurrent HSV. Conversely, defining the mechanism of retrograde transport of HSV from epidermis to DRG, could assist in the current efforts to develop HSV as a gene therapy vector for the treatment of disorders of the peripheral and central nervous system. In this review of HSV (and alphaherpesvirus) transport, it is important to note that during viral transport predominantly in the anterograde direction the virus frequently reverses direction and similarly for transport predominantly in the retrograde direction. This is termed bi-directional transport of viruses which also occurs with organelles.

Structure of herpes simplex virions

The herpes simplex virion has four components: an electron dense core containing the double stranded DNA genome (152 kb), the capsid, the tegument and an outer envelope containing glycoproteins [2]. The icosadeltahedral capsid encloses the DNA core and consists of 162 capsomeres with six different viral proteins (VPs) present on the surface [2]. The surrounding tegument contains 22 proteins and the envelope contains 16 membrane proteins, including 12 different glycoproteins [8] (Table 1).

VIRAL ENTRY AND RETROGRADE TRANSPORT

The size and complexity of eukaryotic cells effectively restricts free intracellular diffusion for molecules larger than Mr 500 000, requiring cells to have organised transport systems. In many cases, viruses utilise these intracellular transport mechanisms to complete their replication cycle [10]. For example transport is more important for viruses that require access to the cell nucleus to replicate, such as herpesviruses and retroviruses,

Table 1. Structural proteins of HSV

Capsid	Tegument	Envelope
pUL6	pUL4	pUL1 (gL)
pUL18 (VP23)	pUL11	pUL10 (gM)
pUL19 (VP5)	pUL13	pUL20
pUL25	pUL14	pUL22 (gH)
pUL35 (VP26)	pUL16	pUL27 (gB)
pUL38 (VP19C)	pUL17	pUL43
	pUL21	pUL44 (gC)
	pUL36 (VP1/2)	pUL45
	pUL37	pUL49.5 (gN)
	pUL41	pUL53 (gK)
	pUL46 (VP11/12)	pUS4 (gG)
	pUL47 (VP13/14)	pUS5 (gJ)
	pUL48 (VP16)	pUS6 (gD)
	pUL49 (VP22)	pUS7 (gI)
	pUL51	pUS8 (gE)
	pUL56	pUS9
	pUS2	
	pUS3	
	pUS10	
	pUS11	
	ICP0	
	ICP4	

The viral genes are designated according to their position in the unique long (U_L) and unique short regions (U_S) of the viral genome with the encoded viral proteins designated with the prefix 'p' [9]. Alternative viral protein (VP) names are given in brackets. ICP, infected cell protein.

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than for viruses that replicate in the cytoplasm, such as paramyxoviruses and *Picornaviridae*. It has been estimated that an HSV-1 capsid would take approximately 230 years to travel 1 cm through the cytoplasm by diffusion alone [11]. During infection of flat cells *in vitro*, it may be possible for HSV-1 capsids to reach the nucleus by diffusion, since the nucleus is only a few microns away from the cytoplasm at the apical surface [4]. However, the slow rate of diffusion is clearly not compatible with viruses travelling very long distances along sensory neurons to establish infection in humans.

Long distance intracellular movement of organelles and vesicles is driven by kinesin and dynein motor proteins which transport cargo along microtubules (MTs). MTs are long cytoskeletal filaments with their fast growing plus-ends usually pointing towards the plasma membrane and in neuronal axons towards the nerve terminals. The MT minus-ends are attached to the MT-organising centre (MTOC) located close to the nucleus. The majority of kinesins transport cargo towards the plus-ends of MTs (anterograde transport) whereas dyneins transport cargo towards the minus-ends (retrograde transport) (Figure 1).

Molecular motor involved in retrograde transport

Cytoplasmic dynein

Cytoplasmic dynein is a minus-end directed, MT-associated molecular motor responsible for retrograde transport in neuronal axons and other cells (Figure 1) [12]. The large (Mr 1.2×10^6) cytoplasmic dynein 1 complex contains heavy chains, intermediate chains, light intermediate chains and light chains (Figure 2) and has been the subject of several recent reviews [13–16]. Cargo-binding specificity depends on varying combinations of intermediate and light chains, the expression of which varies between tissues. Cytoplasmic dynein is also involved in other cellular processes including mitosis, maintenance of the Golgi apparatus and transport of vesicular tubular complexes from the ER to the Golgi [14,17].

Dynein cofactor: dynactin

The dynactin complex is another large, multimolecular complex involved in rapid, MT-associated transport [35]. It is essential for mitosis in multicellular organisms, and thus for viability, and is intimately associated with dynein [35]. It

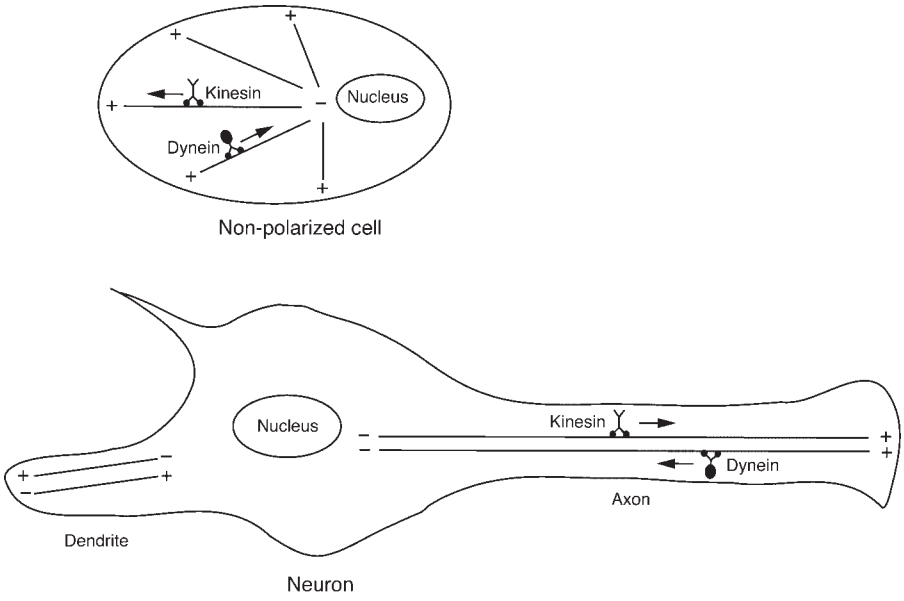

Figure 1. Organisation of microtubules (MTs) in polarised versus non-polarised cells showing the relative MT-dependent movement of the molecular motors dynein and kinesin. In all cells, dynein mediates transport along MTs from the plus (+) or growing end to the minus-end (−) whilst the majority of kinesins mediate transport in the opposite direction. In non-polarised cells, the MTs are anchored at their minus-ends (−) to the perinuclear MT-organising centre. In polarised neurons, the minus-ends of MTs are anchored in the cell body with the plus-end located at the distal end of the axon. Neuronal dendrites contain MTs with a mixed polarity. Kinesin is responsible for anterograde axonal transport while dynein is responsible for retrograde axonal transport

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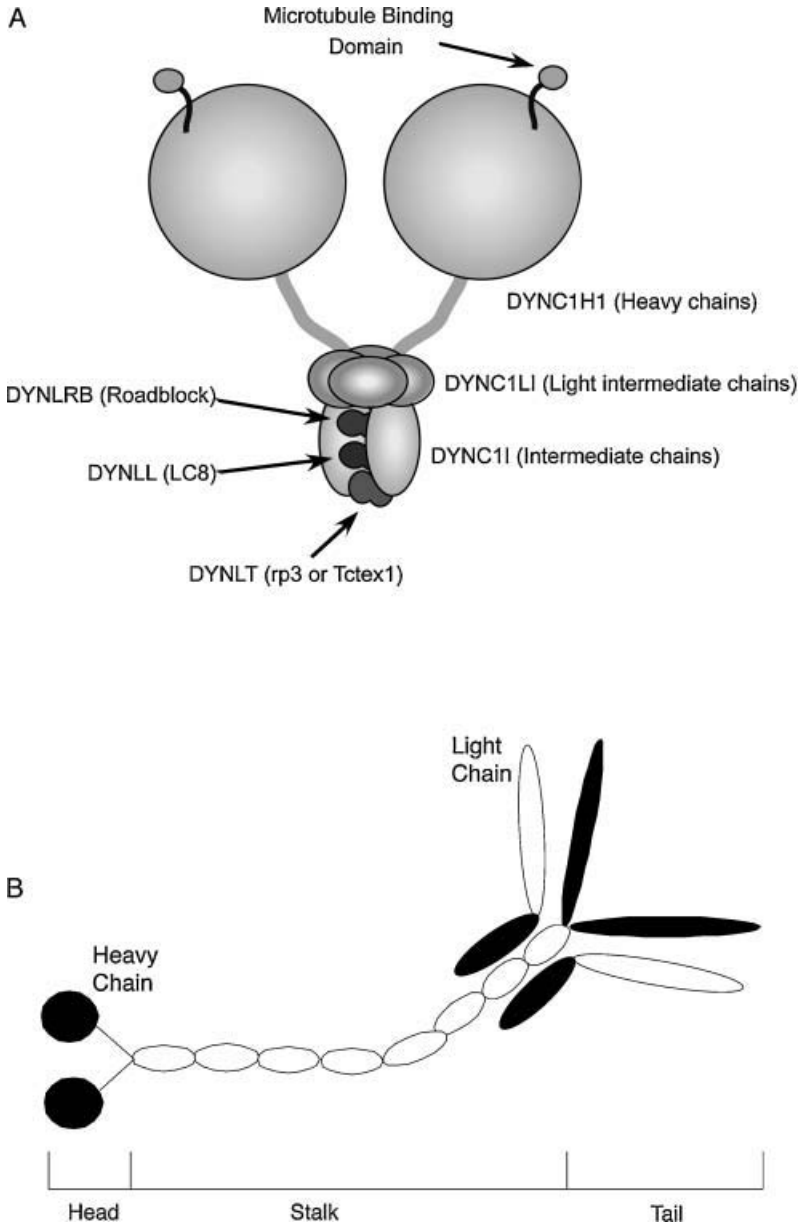

Figure 2. Diagrammatic view of (A) cytoplasmic dynein 1 and (B) kinesin-1. (A) Cytoplasmic dynein 1 heavy chain (DYNC1H1), binds to MTs, and uses ATP to provide motive force for the dynein complex [14]. Cytoplasmic dynein 1 intermediate chain (DYNC1I) forms the scaffold of the dynein complex, is present as a dimer and is thought to contribute to cargo binding [14]. Cytoplasmic dynein 1 light intermediate chains (DYNC1LI1 and 2) contain ATP-binding sites, undergo post-translational phosphorylation, and may be involved in controlling dynein activity [18,19]. Light intermediate chains bind directly to heavy chain, whereas the light chains (each present as two copies) bind to intermediate chain. Cytoplasmic dynein light chain LC8 (DYNLL1 and 2) is thought to bind to cargo and to stabilise the dynein complex [14,20,21]. Cytoplasmic dynein light chain roadblock isoforms (DYNLRB1 and 2) are capable of forming homo and heterodimers [22]. Cytoplasmic dynein light chain Tctex1 (DYNLT) family, either Tctex1 (DYNLT1) or rp3 (DYNLT3), which share 55% amino acid homology [14], are mutually exclusive in cytoplasmic dynein complexes [23]. Despite their similarities, Tctex1 and rp3 exhibit significant differences in cellular and tissue distributions, as well as in their binding specificities [23–30]. (B) Kinesin-1 consists of two identical heavy chains and two identical light chains. Kinesin-1 contains three domains including the MT-binding ATPase head, the stalk and the cargo-binding tail. The stalk consists of heavy chain homodimers as well as heavy and light chain heterodimers formed via heptad-repeat regions in the N-terminal half of light chain and the C-terminal end of the stalk domain of heavy chain [31–33]. Both the heavy and light chain regions which contribute to the tail domain bind a range of proteins that are either themselves cargo or adaptors for binding of cargo [34]

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has recently been shown that dynactin binds directly to MTs and enhances the average length of dynein-driven movements [36,37]. Dynactin is also thought to help in the regulation of dynein activity [38,39] but appears not to be required for attachment of dynein to membranes [40].

Retrograde transport of HSV-1

Herpesviruses infect neuronal cells only by pH-independent fusion of the viral envelope with the plasma membrane while in non-neuronal cells they can enter also via a pH-dependent [41,42] or pH-independent endocytic pathway [43]. Once inside the cell, most HSV-1 tegument proteins are lost, leaving the capsid, with some associated tegument, to be transported to the cell nucleus [44–46]. Unenveloped capsid must first traverse the actin-rich cortex of the host cell, a poorly understood process that seems to involve actin microfilaments, and may be stimulated by phosphorylation of host cell proteins [4,41,47].

Retrograde transport of HSV-1 in axons has been visualised using time-lapse fluorescent microscopy [48]. This transport requires intact MTs, and can be disrupted by colchicine, vinblastine or nocodazole, in both non-polarised cells [49] and sensory neurons [50]. Incoming HSV-1 capsids associate with cytoplasmic dynein and dynactin, and their transport to the nucleus is dynein dependent, since it can be blocked by the over-expression of the dynactin subunit p50 (dynamitin) [51].

Until recently, it was not known which VPs were involved during retrograde HSV transport. The first HSV-1 protein shown to bind to dynein was pUL34, which interacted *in vitro* with an N-terminal fragment of cytoplasmic dynein 1 intermediate chain (DYNC1I)1a [52]. The HSV-1 viral helicase pUL9 contains the typical LC8-binding motif KSTQT [53], and was recently shown to bind to LC8 *in vitro* [54]. Despite these findings, pUL34 and pUL9 are unlikely to play significant roles during retrograde transport of HSV-1. Neither is present in mature HSV-1 virions [55,56], and pUL34 is not found in mature virions of the related PrV [57–59]. Furthermore, deletion of the *UL34* gene does not prevent infection of cells by HSV-1 [60]. In fact, it is hard to envisage what role any of these interactions with dynein would play in the replication of HSV-1. One explanation may be that dynein plays a role in the nuclear import of pUL34 and pUL9, as observed for other VPs [61].

Since the HSV-1 virion loses its envelope and most of the tegument proteins following cell entry, the most likely VPs to interact with dynein or dynactin are those located in the outer capsid or inner tegument. Recent work in our laboratory suggests that the HSV-1 outer capsid protein pUL35 (also designated VP26) is important for retrograde viral transport [62]. The capsid protein pUL35 was shown to interact with Tctex1 and rp3, both in a yeast two-hybrid system and *in vitro*, and the interaction was confirmed using recombinant HSV-1 capsids and intact dynein complexes. The functional significance of this interaction was demonstrated by microinjecting recombinant HSV-1 capsids into live cells. Capsids containing pUL35 colocalised with MTs, Tctex1 and rp3, and moved towards the cell nucleus over time. No movement towards the nucleus was observed for capsids not containing pUL35 [62].

Although pUL35 is not essential for HSV-1 replication *in vitro* [63], its role during viral transport *in vivo* remains to be determined. In one previous study, the role of pUL35 in retrograde transport of HSV-1 to, and replication in, trigeminal ganglia was dissected in a mouse eye model [63]. Deletion of pUL35 decreased the amount of infectious virus in trigeminal ganglia by 100-fold, whereas titres in cell culture (where retrograde transport is less critical) were decreased only twofold. Simultaneous deletion of thymidine kinase (and thus replication) ablated the effects of pUL35 deletion at 72 h after ocular infection, suggesting that the virus could still be transported in the absence of pUL35. However, earlier time points may be needed to detect a decrease in transport velocity or efficiency, since wild-type virus can arrive as early as 19 h post-infection [64].

Recently, two studies with both PrV and HSV-1 lacking pUL35 have shown no significant effect on dynein-dependent retrograde viral transport in cell culture [65,66]. The relative contribution of other tegument or capsid proteins to HSV-1 transport is not known, although it has been suggested that the major tegument protein pUL36, or one of the minor capsid proteins such as pUL25, may also contribute to dynein binding *in vivo* [51,67]. In the case of PrV, the so-called inner tegument proteins pUL36, pUL37 and pUS3 have been shown to remain associated with the capsid during entry

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[68,69]. In addition, removal of outer tegument proteins to expose inner tegument proteins has been shown to enhance the *in vitro* MT-dependent transport of HSV-1 capsids [70]. These tegument proteins are therefore the likely complementary candidates to pUL35 for engaging the dynein/dynactin complex [65,66,70]. In the case of pUL36 and pUL37, our laboratory has failed to detect a direct interaction with the subunits of dynein using the yeast two-hybrid assay [62]. However, further broader investigations are required to establish whether or not pUL36, pUL37 and pUS3 are involved in binding of the dynein/dynactin complex. Binding of these or other PrV or HSV-1 proteins to dynein implies redundancy in VPs available for retrograde transport, which is not surprising for such an important stage during viral replication. There are precedents for functional redundancy in other HSV-1 and PrV proteins [71,72].

ANTEROGRADE TRANSPORT AND VIRAL EGRESS

HSV-1 spread and transmission is highly dependent on the MT-mediated transport of the virus from the neuronal cell body to the axon tips. Kinesins are known to drive the intracellular transport of organelles, vesicles and viruses to the cell periphery and, in the case of neuronal axons, to the axon termini (Figure 1).

Molecular motor involved in anterograde transport

Kinesin

The kinesin superfamily (KIF) of molecular motor proteins, which number 45 in mammalian cells together with cytoplasmic dynein are responsible for MT-dependent transport of cargo in eukaryotic cells [12,73–76]. Kinesins are classified according to the position of the highly conserved motor domain (binds MTs) which may be N-terminal (N-kinesin), middle (M-kinesin) or C-terminal (C-kinesin) [75,77,78]. The majority of kinesins are N-kinesins which transport cargo such as membranous vesicles, protein complexes, mRNA and viruses towards the plus or growing end of MTs [73–75]. In the case of neuronal cells, this corresponds to anterograde transport, that is away from the cell body towards the axon tip (Figure 1).

The members of the N-kinesin subfamily known to be involved in transport of cargo down axons (and therefore likely to transport HSV-1) include kinesin-1 (KIF5 or conventional kinesin), kinesin-2 (KIF3) and kinesin-3 (KIF1) [74]. Kinesin-1, the first kinesin to be discovered [79], is a tetrameric protein consisting of two identical heavy chains of Mr 120 000 and two identical light chains of Mr 64 000 (Figure 2) [80]. The mammalian genome contains three kinesin-1 heavy chain (KHC) genes (*KIF5A*, *KIF5B* and *KIF5C*) and three kinesin-1 light chain (KLC) genes (*KLC1*, *KLC2*, *KLC3*) [12]. *KIF5A* and *KIF5C* are expressed only in neurons while *KIF5B* is ubiquitously expressed in all tissues.

It is becoming apparent that both KHC and KLC are capable of interacting with multiple cargo typically via adaptor or scaffolding proteins [34,81]. Several cellular (and homologous) proteins have now been identified which bind to the tail domain of kinesin-1 (Figure 2) either via the C-terminal tetratricopeptide repeat (TPR) region of KLC [34,74] or to the heptad-repeat region of the C-terminal heavy chain tail domain [82,83].

Anterograde transport of HSV-1

Non-neuronal cells

After assembly in the nucleus, the mature HSV-1 (DNA-containing) nucleocapsid undergoes primary envelopment through the inner nuclear membrane into the perinuclear space [8,9]. The enveloped particles are then deenveloped at the outer nuclear membrane. This is followed by the addition of inner tegument onto the capsid before acquisition of outer tegument and secondary envelopment, at the trans-Golgi [8,84]. Fully assembled virions are finally released by exocytosis. In non-polarised cells, MTs radiate from the MTOC, located usually near the nucleus, with their minus-ends attached to the MTOC and the plus-ends extending to the periphery of the cell (Figure 1). Kinesin is presumably important for the transport of enveloped virus from the Golgi to the cell surface given that the polarity of the MTs requires a plus-end directed motor [7,17]. In the case of polarised non-neuronal cells, MTs are arranged in an apical–basal direction with their minus-ends pointing towards the apical surface and the plus-ends pointing towards the basal surface. Kinesin would presumably mediate exit towards the basal surface. The precise kinesin(s)

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involved in either process have not yet been defined. A recent study on PrV has shown that the major tegument protein pUL36 is required for MT-dependent transport of capsid during egress from non-polarised cells [85]. Whether pUL36 recruits kinesin at all and whether this is direct or indirect remains to be determined. One possible link could be via the KIF5B-binding protein p180 which also interacts with the homologue of pUL36 in human cytomegalovirus [85–87].

Properties of neuronal cells

HSV-1 infects DRG neurons. These neurons are ensheathed by closely applied satellite cells, analogous to Schwann cells, and are separated by support cells, mainly fibroblasts. DRG neurons only have axons which bifurcate outside the DRG to eventually innervate the spinal cord and epithelium. The neurons consist of a cell body joined to the axon by the axon hillock. The axon hillock is the very specialised neck region which regulates electrical excitability and specialised features required for axon function. It contains key organelles and a specialised dense cytoskeletal organisation required for sorting axonal transport of protein and mRNA. Certain organelles such as Golgi and lysosomes accumulate in the cell body prior to the hillock, but synaptic vesicles and mitochondria are free to pass this compartmental boundary [88]. The axon hillock also provides a functional barrier to diffusion of plasma membrane molecules between axon and cell body, maintaining differences between membrane constituents within the two lipid compartments [88]. When cultured *in vitro*, with added nerve growth factor, axons are tipped by growth cones, expanded regions which consist of a central core of vesicles of heterogenous structure and distal filopodia containing a core of MTs and surrounding actin network. The axons branch at the site of the growth cone and extend, leaving varicosities adjacent to or within the bifurcations.

Transport along axons

Egress of HSV-1 directly from the neuronal cell body follows a similar pathway to that in non-neuronal cells (Figure 3) [4,89,90]. However, two models have been proposed for anterograde axonal transport of HSV-1. The subassembly model proposes the separate transport of viral capsids and glycoproteins with assembly and release by exocytosis either along the axon shaft or at the axon termini (Figure 3, path A) [90–102]. The alternative 'married' model proposes that only fully assembled enveloped virions move down axons in vesicles (Figure 3, path B) [103,104].

Prior to 1994, knowledge of the mechanism of anterograde axonal transport of HSV-1 was confined to occasional electron microscopy profiles of HSV or PrV in animal models (Table 2). Then a two-chamber DRG neuron–skin centre system was developed in our laboratory to examine this process *in vitro*. Using confocal microscopy (CFM), transmission electron microscopy (TEM) and the MT assembly inhibitor nocodazole, only unenveloped capsids were observed to be transported along MTs of human fetal DRG neuronal axons *in vitro* at a rate compatible with MT-associated rapid axonal transport [91]. Subsequently, using transmission immunoelectron microscopy (TIEM) with dual immunogold labelling, the capsids were found to be coated with tegument proteins, especially pUL48 during transport [92]. Glycoproteins were transported separately within axonal vesicles [92]. These results confirmed our original hypothesis that HSV-1 is transported anterogradely in the axon as two components, prior to distal assembly (Figure 3).

A complementary approach, involving cultures of dissociated rat DRG neurons and high multiplicity inputs of virus, was also used to follow the retrograde transport of virions to the nucleus and subsequent appearance, distribution and kinetics of capsid, tegument and glycoprotein antigens throughout the cell body and anterograde transport into the axons [93]. Furthermore, brefeldin A inhibited the transport of glycoproteins into axons but not of capsids, again providing evidence for the subassembly hypothesis [93].

Subsequently, most of these observations have been confirmed by a number of other groups for HSV but not for PrV, for example the use of an *in vitro* mouse eye model has also demonstrated separate axonal transport of HSV-1 glycoproteins from capsids [99–101]. Nevertheless, using PrV labelled with green fluorescent protein (GFP) a similar velocity of anterograde transport to HSV [105] and a lack of colocalisation of PrV envelope proteins with capsids in chick neuronal axons was initially reported [97]. The transport of capsids but not glycoproteins into the axons in PrV mutants

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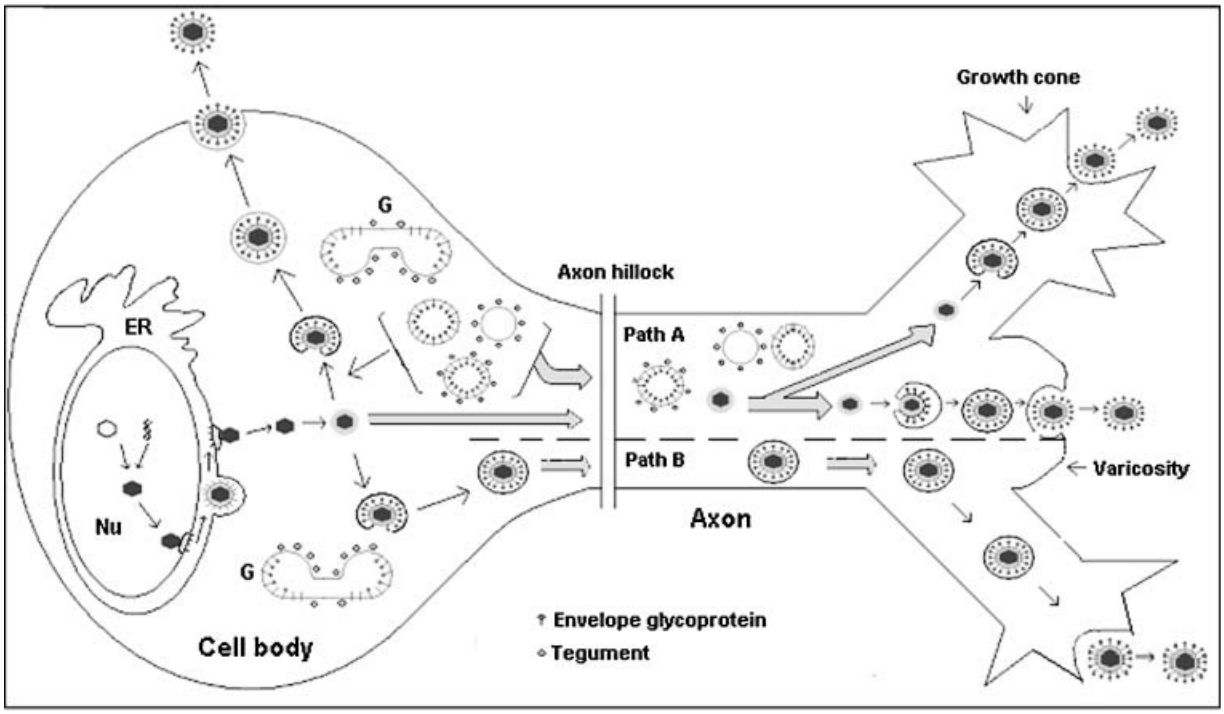

Figure 3. Schematic diagram of the proposed model for assembly and egress of HSV-1 from neuronal cells. Enveloped virions can exit the cell body directly in a similar fashion to the way they exit non-neuronal cells (upper left hand portion of figure). Alternatively, virions can be transported in an anterograde direction down axons via two proposed paths. Path A: illustrates the subassembly model. Unenveloped capsids coated by inner tegument proteins are transported from the cell body into axons, accumulating in varicosities or growth cones. At these sites, capsids invaginate vesicles, acquiring tegument and envelope proteins. These enveloped capsids, now contained within vesicles, exit locally via exocytosis. Path B: illustrates the 'married' model. Enveloped HSV-1 capsids from the cell body may possibly also enter the mid- and distal regions of the axon and exit via the varicosities and growth cones. G, Golgi; Nu, nucleus. Adapted with permission from [90]

deleted of the protein pUS9 was also initially thought to support the subassembly hypothesis for PrV [96].

Exit from growth cones/varicosities

In recent studies of transport, assembly and egress of HSV-1 in mid- and distal axons of infected explanted human fetal DRG, we showed that capsid (pUL19) and tegument (pUL37) proteins were colocalised with envelope (gG) proteins in axonal varicosities and in growth cones at 24 and 48 h p.i. by CFM [90]. Varicosities are dilations of axons containing high concentrations of vesicles and few MTs, usually at axonal branch points, representing residual growth cones left behind by growth of the branching axonal termini. TEM of longitudinal sections of axons *in situ* showed enveloped and unenveloped capsids in the axonal varicosities and growth cones, whereas in the mid-region of the axons, predominantly unenveloped capsids were observed. Partially enveloped capsids, apparently budding into vesicles, were observed in axonal varicosities and growth cones, but not during viral attachment and entry into axons. Tegument proteins (pUL49) were found associated with vesicles in growth cones, either alone or together with envelope (gD) proteins, by TIEM. Extracellular virions were observed adjacent to axonal varicosities and growth cones, with some virions observed in crescent-shaped invaginations of the axonal plasma membrane, suggesting exit at these sites. Thus, these findings suggested that varicosities and growth cones are probable sites of HSV-1 envelopment of at least a high proportion of virions in the mid- to distal axon. Envelopment probably occurs by budding of capsids into vesicles with associated tegument and envelope proteins. Virions appear to exit from these sites by exocytosis perhaps utilising

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Table 2. Identification of alphaherpesvirus virions in axons by TEM or TIEM

Reference	System	Site of viral particles in axon	Observations
Enveloped virions			
[109]	HSV–chick embryo DRG	Uncertain	No virions in axons
[110]	HSV–rabbit optic nerve (retinal ganglia)	Uncertain	Enveloped virions in cisternae (ER); occasional unenveloped capsids
[111]	HSV–mouse DRG	Proximal	Single enveloped virion; no MTs visible
[112]	HSV–mouse DRG, coeliac ganglion	Proximal uncertain	Periaxonal virions; Atypical particles in vesicle-rich region
[113]	HSV–dissoc rat DRG; *in vitro*	Neuritic extension	Three enveloped virions; no MTs visible
[114]	PrV–rat CNS sensory vagal processes	Uncertain	Single enveloped virions; no MTs visible
[115]	HSV–mouse trigeminal ganglion	? Proximal	Several enveloped virions; no MTs visible
[103,104]	PrV–sympathetic ganglia	Proximal (and axon hillock)	Many enveloped virions
[123,125]	PrV–sympathetic ganglia	Mid-axon	Several enveloped virions
Unenveloped virions			
[91]	HSV–DRG, two chamber system	Mid-distal	Capsids surrounded by MTs
[93]	HSV–DRG, two chamber system	Mid-distal	Capsids surrounded by MTs; VP5-VP16 colocalisationon particles but not with gB by TIEM
[93]	HSV–dissoc rat DRG; *in vitro*	Uncertain	VP5-labelled capsid in axon by TIEM
[89]	HSV–dissoc DRG	Uncertain	Capsid labelled for VP16 in axon
[95]	HSV–mouse trigeminal ganglion	Mid	Unenveloped capsids identified by TIEM and adjacent to MTs
[104]	PrV–sympathetic ganglia	Proximal	Two unenveloped capsids after inhibition with brefeldin A
[100]	HSV–mouse retinal ganglia	Mid-axon	Unenveloped capsids (VP5 immunolabelled); (?) enveloped virions
Both enveloped and unenveloped virions			
[116]	HSV–suckling mice, spinal cord	Uncertain	Single unenveloped capsid in unmyelinated axon; cluster of enveloped virions in cisternae (ER) (myelinated axon)
[117]	PrV–mice DRG	Proximal	Several enveloped virions in vesicle-rich region
	PrV–sciatic nerve	Mid	Enveloped and unenveloped virions (not shown)
[90]	HSV–DRG *in vitro*	Mid-distal axon, growth cones, varicosities	Enveloped and unenveloped capsids in varicosities and growth cones (and adjacent axon)

MT, microtubules; TEM, transmission electron microscopy; TIEM, transmission immuno-electron microscopy; DRG, dorsal root ganglion; ?, uncertain observation. Adapted with permission from Reference [90].

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normal processes of neurotransmitter exocytosis. Similar varicosities have been observed to appear *de novo* after infection of swine trigeminal ganglionic neurons with PrV and act as viral exit sites [106].

Anterograde axonal transport of enveloped pseudorabies virions

Several recent reports from two related groups have challenged this 'separate transport' or 'subassembly' hypothesis for anterograde transport of alphaherpesviruses. Del Rio *et al.*, using dual monomeric red fluorescent protein (mRFP)-pUL35/GFP-pUL49-labelled PrV in cultured rat superior cervical ganglion neurons, showed heterogeneity in GFP-labelled pUL49 fluorescence that was similar in purified virions, the cell body and axons of neurons [104]. They also showed large numbers of enveloped particles in the proximal axon. The transport of these enveloped capsids was inhibited by brefeldin A, leaving a small number of unenveloped capsids within the proximal axon. In a three-chamber system, enveloped particles within vesicles were also observed within the mid-axon [103]. More recent studies with dual-labelled glycoprotein and capsid showed coincident anterograde transport [107]. Capsid label alone only occurred during apparent retrograde phases of the nett anterograde movement. This study acknowledges the likely presence of unenveloped capsids in sensory axons infected by PrV but suggests they are incidental retrogradely transported particles. However, the likely site of unenvelopment was not discussed and such a hypothesis does not explain the findings for HSV in varicosities.

The controversy about the anterograde axonal transport and exit of alphaherpesviruses

The apparently contradictory findings for anterograde transport of HSV or PrV in axons (summarised in Table 3) have now been demonstrated in five different laboratories, three for HSV and two for PrV. The variables which might explain these findings, include differences between the two alphaherpesviruses, different types of neurons, different peak kinetics of transport or technical differences. Although both of the herpesviruses share similar mechanisms of retrograde transport and might be expected to demonstrate similar mechanisms of anterograde transport, the two viruses show around 30% protein sequence homology (based on the comparison of structural proteins [59,72,108]). Hence, there may be quantitative or qualitative differences in their transport. Although the earlier studies of PrV were conducted in chick sympathetic axons, more recent studies have utilised sensory axons. Similarly, different types of neuronal systems have been used for HSV and PrV, including sympathetic, trigeminal and DRG neurons and murine eye models. Interestingly, the peak kinetics of PrV anterograde axonal transport appears to be much earlier (10–13 h p.i.) than with HSV (15–28 h p.i.). Different study techniques have been utilised such as serial fixation and CFM, real-time fluorescence with single or dual labelling of viruses, TEM in transverse or longitudinal section and TIEM. However, in

Table 3. Controversies in the anterograde axonal transport of alphaherpesviruses (HSV and PrV)

Unenveloped HSV capsids predominate in axons versus enveloped PrV virions in proximal sympathetic axons
Latest suggested explanation for the presence of enveloped capsids in axons is that envelopment of (HSV) virions occurs within varicosities (as shown by the presence of partially enveloped virions) [90]
Separate HSV capsid and glycoprotein antigen in regions of axons between varicosities and growth cones versus co-transport in axons of PrV capsid and glycoprotein antigens
Latest suggested explanation for colocalised versus separate capsid and envelope proteins is that enveloped (PrV) virions are transported anterogradely whereas unenveloped capsids are transported retrogradely (but there is no explanation of how the unenveloped capsids are generated in the mid-axon) [107]

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recent studies similar techniques (i.e. live imaging) have been used without resolution of the controversy. These immunofluorescent and ultrastructural techniques have their advantages and disadvantages but complement each other. They range from demonstrating coincident transport of two antigens with real-time fluorescence to the much greater sensitivity and ability of TEM or TIEM to demonstrate capsids with or without label (to ensure differentiation from other axonal structures). At the time of writing this review, the controversy remains unresolved. Clearly, exact duplication of the technologies for studying the two viruses is required before it can be accepted that there may be fundamental differences in the mechanisms of anterograde transport for these two viruses.

Kinesin–viral protein interactions

The mechanism of axonal transport of HSV or PrV in a nett anterograde direction obviously has implications for the mechanism of kinesin-driven MT-associated anterograde transport. If capsid coated with some tegument is transported separately, then the opportunity exists for a direct role of tegument and/or capsid in recruiting the kinesin transport machinery. Alternatively, if only enveloped virions are transported then either a kinesin-cellular receptor or viral glycoprotein cytoplasmic tail would provide the link with the cellular transport machinery. Work from our laboratory has previously identified the HSV-1 tegument protein pUS11 as a KIF5B-binding protein [118]. The interaction was dependent on the C-terminal domain of pUS11 and the cargo-binding domain of KIF5B. Presumably, the role of this interaction would be in anterograde axonal transport of unenveloped capsids. Certainly, KIF5B was shown to be present by TIEM on such viral subparticles in human DRG axons [118]. In addition, by immuno-CFM, pUS11 was shown to be co-transported with capsids down axons [118]. Interestingly, pUS11 has also been shown to interact with a cellular KLC-like protein, PAT-1 [119]. In this case, the precise role of this interaction is unclear, as PAT-1 has no obvious motor domain itself nor does it appear to associate with KIF5B (unpublished observations from our laboratory).

Like retrograde transport, the identified pUS11/KIF5B interaction is probably redundant in anterograde transport. In cell lines [120] and from the cell body of neurons (unpublished observations from our laboratory) deletion of the *US11* gene has no effect on viral egress. This is not unexpected, as the form of virus transported to the plasma membrane from the Golgi would be enveloped. In this case, pUS11 would not be accessible to kinesin and most likely a kinesin-cellular receptor or viral glycoprotein cytoplasmic tail would be required for transport. This is supported by a recent study which showed KIF5B associated with enveloped HSV-1 also containing the known kinesin cellular receptor, amyloid precursor protein (APP) [121]. Furthermore, pUS11 is not conserved across the closely related alphaherpesviruses, implying that other viral tegument/capsid proteins are recruited for axonal transport of unenveloped capsids. Even in the case of HSV-1, more than one kinesin may be involved in axonal transport and as such other VPs are likely to be involved. A recent study has identified a direct interaction between the HSV-2 membrane-associated tegument protein pUL56 and KIF1A [122]. This interaction may be important for anterograde axonal transport of viral glycoprotein-containing vesicles. A series of studies using deletion mutants of HSV-1 and PrV that do not express pUS9 or gE have also supported a role for these VPs in anterograde axonal transport [96,101,123–125]. To date, a direct link between either pUS9 or gE and kinesin has not been established.

BI-DIRECTIONAL VIRAL TRANSPORT

Transport of herpesviruses and adenoviruses has been shown to be bi-directional. With adenovirus, there is an apparent competition between plus-end and minus-end-directed molecular motors [126,127]. Direct interaction of DYNC1I and KLC also implies that kinesin bound to cargo can recruit and coordinate the activity of dynein and *vice versa* [128]. Measurements in real-time of GFP-pUL35-labelled PrV in chick DRGs indicates that herpesvirus capsid transport during entry and egress, at least within axons, is also bi-directional [105,129]. For PrV entry, movement was observed in both directions but the speed of retrograde transport was greater than anterograde transport resulting in net movement up the axon [129]. For egress, retrograde transport was the same speed as entry (suggesting the same motor was recruited, i.e. dynein) while anterograde transport was now faster resulting in nett move-

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ment down the axon [105,129]. It was concluded that the activity of the anterograde motor (assuming the same kinesin was recruited during entry and egress) is directly regulated by the viral cargo [129] as has been suggested for cellular cargo [130]. Alternatively, if the viral capsid/tegument structure does vary in composition during entry and egress, as appears to be the case for HSV-1 and PrV, then different kinesins, with different rates of transport, may be recruited during entry and egress. Recently, the change in tegument composition of PrV between entry and egress has been shown to determine the direction of axonal movement of capsids [69]. Retrograde transport was associated with capsids containing the inner tegument proteins pUL36 and pUL37 whilst the addition of the outer tegument proteins pUL47, pUL48 and pUL49 was associated with anterograde transport. Elucidation of which kinesins are recruited and the composition of HSV-1 during entry and egress will aid in further refining the current model for transport of herpesviruses.

CONCLUSION

The mechanisms of retrograde and anterograde transport of alphaherpesviruses, including HSV and PrV, in neuronal axons is progressing. For retrograde transport, there is a reasonable consensus that after fusion of the virus at the axolemma most of the tegument proteins are lost. For PrV, only pUL36/pUL37 and pUS3 have been identified on the incoming capsid in cell lines and only pUL36/pUL37 in axons. For HSV, most of the outer tegument proteins have been shown to be phosphorylated and lost but this has not been as precisely defined as for PrV within cell lines or axons. The VPs involved in transport remain controversial. The capsid protein pUL35 has been shown to bind dynein light chains and to be essential for retrograde transport of the capsid to the nuclear membrane, but this protein is not essential for transport of the whole virion. This suggests a redundant process with one of the inner tegument proteins pUL36 or pUL37 being the most likely candidates for the complementary-binding proteins for dynein.

The mechanism of anterograde transport of alphaherpesviruses is highly controversial. There is marked disagreement between laboratories studying HSV transport in DRG sensory neurons and the groups studying PrV in chick sympathetic and sensory neurons. Using predominantly real-time fluorescence with dual-labelled virus, PrV seems to be transported anterogradely as whole virus particles and enveloped virions have been shown to predominate in sympathetic axons. For HSV, unenveloped capsids have been shown to predominate in the regions of axons between branched points and varicosities where enveloped virions and partially enveloped virions have been identified by electron microscopy. In these, same stretches of axon capsid and glycoprotein do not usually colocalise.

Whether there is truly a difference in the mechanism of anterograde transport for the two alphaherpesviruses HSV and PrV is unclear. Further studies using congruent technologies are needed to resolve these controversies and to determine whether there are similar or different mechanisms of transport between HSV and PrV and whether these are reflected in other alphaherpesviruses such as VZV.

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REFERENCES

1. Cunningham AL, Dwyer DE. The pathogenesis underlying the interaction of HIV and herpes simplex virus after co-infection. *J HIV Ther* 2004; **9**: 9–13.
2. Roizman B, Sears AE. Herpes simplex viruses and their replication. In *Fields Virology*, Fields BN, Knipe DM, Howley PM (eds). Lippincott-Raven: Philadelphia, 1996; 2231–2295.
3. Dohner K, Sodeik B. The role of the cytoskeleton during viral infection. *Curr Top Microbiol Immunol* 2005; **285**: 67–108.
4. Garner JA. Herpes simplex virion entry into and intracellular transport within mammalian cells. *Adv Drug Deliv Rev* 2003; **55**: 1497–1513.
5. Smith GA, Enquist LW. Break ins and break outs: viral interactions with the cytoskeleton of Mammalian cells. *Annu Rev Cell Dev Biol* 2002; **18**: 135–161.
6. Dohner K, Nagel CH, Sodeik B. Viral stop-and-go along microtubules: taking a ride with dynein and kinesins. *Trends Microbiol* 2005; **13**: 320–327.
7. Radtke K, Dohner K, Sodeik B. Viral interactions with the cytoskeleton: a hitchhiker's guide to the cell. *Cell Microbiol* 2006; **8**: 387–400.

8. Mettenleiter TC. Budding events in herpesvirus morphogenesis. *Virus Res* 2004; **106**: 167–180.
9. Mettenleiter TC. Intriguing interplay between viral proteins during herpesvirus assembly or: the herpesvirus assembly puzzle. *Vet Microbiol* 2006; **113**: 163–169.
10. Greber UF, Way M. A superhighway to virus infection. *Cell* 2006; **124**: 741–754.
11. Sodeik B. Mechanisms of viral transport in the cytoplasm. *Trends Microbiol* 2000; **8**: 465–472.
12. Goldstein LS, Yang Z. Microtubule-based transport systems in neurons: the roles of kinesins and dyneins. *Annu Rev Neurosci* 2000; **23**: 39–71.
13. Vallee RB, Williams JC, Varma D, *et al*. Dynein: an ancient motor protein involved in multiple modes of transport. *J Neurobiol* 2004; **58**: 189–200.
14. King SM. The dynein microtubule motor. *Biochim Biophys Acta* 2000; **1496**: 60–75.
15. Levy JR, Holzbaur EL. Cytoplasmic dynein/dynactin function and dysfunction in motor neurons. *Int J Dev Neurosci* 2006; **24**: 103–111.
16. Pfister KK, Shah PR, Hummerich H, *et al*. Genetic analysis of the cytoplasmic dynein subunit families. *PLoS Genet* 2006; **2**: 11–26.
17. Murshid A, Presley JF. ER-to-Golgi transport and cytoskeletal interactions in animal cells. *Cell Mol Life Sci* 2004; **61**: 133–145.
18. Gill SR, Cleveland DW, Schroer TA. Characterization of DLC-A and DLC-B, two families of cytoplasmic dynein light chain subunits. *Mol Biol Cell* 1994; **5**: 645–654.
19. Hughes SM, Vaughan KT, Herskovits JS, *et al*. Molecular analysis of a cytoplasmic dynein light intermediate chain reveals homology to a family of ATPases. *J Cell Sci* 1995; **108**: 17–24.
20. Makokha M, Hare M, Li M, *et al*. Interactions of cytoplasmic dynein light chains Tctex-1 and LC8 with the intermediate chain IC74. *Biochemistry* 2002; **41**: 4302–4311.
21. Nyarko A, Hare M, Hays TS, *et al*. The intermediate chain of cytoplasmic dynein is partially disordered and gains structure upon binding to light-chain LC8. *Biochemistry* 2004; **43**: 15595–15603.
22. Nikulina K, Patel-King RS, Takebe S, *et al*. The Roadblock light chains are ubiquitous components of cytoplasmic dynein that form homo- and heterodimers. *Cell Motil Cytoskeleton* 2004; **57**: 233–245.
23. Tai AW, Chuang JZ, Sung CH. Cytoplasmic dynein regulation by subunit heterogeneity and its role in apical transport. *J Cell Biol* 2001; **153**: 1499–1509.
24. Chuang JZ, Milner TA, Sung CH. Subunit heterogeneity of cytoplasmic dynein: differential expression of 14 kDa dynein light chains in rat hippocampus. *J Neurosci* 2001; **21**: 5501–5512.
25. Kai N, Mishina M, Yagi T. Molecular cloning of Fyn-associated molecules in the mouse central nervous system. *J Neurosci Res* 1997; **48**: 407–424.
26. King SM, Barbarese E, Dillman JF III, *et al*. Cytoplasmic dynein contains a family of differentially expressed light chains. *Biochemistry* 1998; **37**: 15033–15041.
27. Nagano F, Orita S, Sasaki T, *et al*. Interaction of Doc2 with tctex-1, a light chain of cytoplasmic dynein. Implication in dynein-dependent vesicle transport. *J Biol Chem* 1998; **273**: 30065–30068.
28. Roux AF, Rommens J, McDowell C, *et al*. Identification of a gene from Xp21 with similarity to the tctex-1 gene of the murine t complex. *Hum Mol Genet* 1994; **3**: 257–263.
29. Tai AW, Chuang JZ, Bode C, *et al*. Rhodopsin's carboxy-terminal cytoplasmic tail acts as a membrane receptor for cytoplasmic dynein by binding to the dynein light chain Tctex-1. *Cell* 1999; **97**: 877–887.
30. Lo KW, Kogoy JM, Pfister KK. The DYNLT3 light chain directly links cytoplasmic dynein to a spindle checkpoint protein, Bub3. *J Biol Chem* 2007; **282**: 11205–11212.
31. Diefenbach RJ, Mackay JP, Armati PJ, *et al*. The C-terminal region of the stalk domain of ubiquitous human kinesin heavy chain contains the binding site for kinesin light chain. *Biochemistry* 1998; **37**: 16663–16670.
32. Verhey KJ, Lizotte DL, Abramson T, *et al*. Light chain-dependent regulation of kinesin's interaction with microtubules. *J Cell Biol* 1998; **143**: 1053–1066.
33. Gauger AK, Goldstein LS. The *Drosophila* kinesin light chain. Primary structure and interaction with kinesin heavy chain. *J Biol Chem* 1993; **268**: 13657–13666.
34. Gindhart JG. Towards an understanding of kinesin-1 dependent transport pathways through the study of protein-protein interactions. *Brief Funct Genomic Proteomic* 2006; **5**: 74–86.
35. Schroer TA. Dynactin. *Annu Rev Cell Dev Biol* 2004; **20**: 759–779.
36. Kamal A, Goldstein LS. Connecting vesicle transport to the cytoskeleton. *Curr Opin Cell Biol* 2000; **12**: 503–508.
37. King SJ, Schroer TA. Dynactin increases the processivity of the cytoplasmic dynein motor. *Nat Cell Biol* 2000; **2**: 20–24.
38. Holleran EA, Karki S, Holzbaur EL. The role of the dynactin complex in intracellular motility. *Int Rev Cytol* 1998; **182**: 69–109.
39. Kumar S, Zhou Y, Plamann M. Dynactin-membrane interaction is regulated by the C-terminal domains of p150Glued. *EMBO Rep* 2001; **2**: 939–944.
40. Haghnia M, Cavalli V, Shah SB, *et al*. Dynactin is required for coordinated bidirectional motility,

DOI: 10.1002/rmv

but not for dynein membrane attachment. *Mol Biol Cell* 2007 **18**: 2081–2089.
41. Qie L, Marcellino D, Herold BC. Herpes simplex virus entry is associated with tyrosine phosphorylation of cellular proteins. *Virology* 1999; **256**: 220–227.
42. Nicola AV, Hou J, Major EO, *et al*. Herpes simplex virus type 1 enters human epidermal keratinocytes, but not neurons, via a pH-dependent endocytic pathway. *J Virol* 2005; **79**: 7609–7616.
43. Milne RS, Nicola AV, Whitbeck JC, *et al*. Glycoprotein D receptor-dependent, low-pH-independent endocytic entry of herpes simplex virus type 1. *J Virol* 2005; **79**: 6655–6663.
44. Morrison EE, Stevenson AJ, Wang YF, *et al*. Differences in the intracellular localization and fate of herpes simplex virus tegument proteins early in the infection of Vero cells. *J Gen Virol* 1998; **79**: 2517–2528.
45. Morrison EE, Wang YF, Meredith DM. Phosphorylation of structural components promotes dissociation of the herpes simplex virus type 1 tegument. *J Virol* 1998; **72**: 7108–7114.
46. Ojala PM, Sodeik B, Ebersold MW, *et al*. Herpes simplex virus type 1 entry into host cells: reconstitution of capsid binding and uncoating at the nuclear pore complex in vitro. *Mol Cell Biol* 2000; **20**: 4922–4931.
47. Wittels M, Spear PG. Penetration of cells by herpes simplex virus does not require a low pH-dependent endocytic pathway. *Virus Res* 1991; **18**: 271–290.
48. Bearer EL, Breakefield XO, Schuback D, *et al*. Retrograde axonal transport of herpes simplex virus: evidence for a single mechanism and a role for tegument. *Proc Natl Acad Sci USA* 2000; **97**: 8146–8150.
49. Sodeik B, Ebersold MW, Helenius A. Microtubule-mediated transport of incoming herpes simplex virus 1 capsids to the nucleus. *J Cell Biol* 1997; **136**: 1007–1021.
50. Topp KS, Meade LB, LaVail JH. Microtubule polarity in the peripheral processes of trigeminal ganglion cells: relevance for the retrograde transport of herpes simplex virus. *J Neurosci* 1994; **14**: 318–325.
51. Dohner K, Wolfstein A, Prank U, *et al*. Function of Dynein and dynactin in herpes simplex virus capsid transport. *Mol Biol Cell* 2002; **13**: 2795–2809.
52. Ye GJ, Vaughan KT, Vallee RB, *et al*. The herpes simplex virus 1 UL34 protein interacts with a cytoplasmic dynein intermediate chain and targets nuclear membrane. *J Virol* 2000; **74**: 1355–1363.
53. Rodriguez-Crespo I, Yelamos B, Roncal F, *et al*. Identification of novel cellular proteins that bind to the LC8 dynein light chain using a pepscan technique. *FEBS Lett* 2001; **503**: 135–141.
54. Martinez-Moreno M, Navarro-Lerida I, Roncal F, *et al*. Recognition of novel viral sequences that associate with the dynein light chain LC8 identified through a pepscan technique. *FEBS Lett* 2003; **544**: 262–267.
55. Reynolds AE, Wills EG, Roller RJ, *et al*. Ultrastructural localization of the herpes simplex virus type 1 UL31, UL34, and US3 proteins suggests specific roles in primary envelopment and egress of nucleocapsids. *J Virol* 2002; **76**: 8939–8952.
56. Roizman B, Knipe DM. Herpes simplex viruses and their replication. In *Fields Virology*, Knipe DM, Howley PM, Griffin DE, Lamb RA, Martin MA, Roizman B, Straus SE (eds). Lippincott Williams & Wilkins: Philadelphia, 2001; 2399–2459.
57. Fuchs W, Klupp BG, Granzow H, *et al*. The interacting UL31 and UL34 gene products of pseudorabies virus are involved in egress from the host-cell nucleus and represent components of primary enveloped but not mature virions. *J Virol* 2002; **76**: 364–378.
58. Klupp BG, Granzow H, Mettenleiter TC. Primary envelopment of pseudorabies virus at the nuclear membrane requires the UL34 gene product. *J Virol* 2000; **74**: 10063–10073.
59. Klupp BG, Granzow H, Mundt E, *et al*. Pseudorabies virus UL37 gene product is involved in secondary envelopment. *J Virol* 2001; **75**: 8927–8936.
60. Roller RJ, Zhou Y, Schnetzer R, *et al*. Herpes simplex virus type 1 U(L)34 gene product is required for viral envelopment. *J Virol* 2000; **74**: 117–129.
61. Moseley GW, Martino Roth D, Dejesus MA, *et al*. Dynein light chain association sequences can facilitate nuclear protein import. *Mol Biol Cell* 2007; **18**: 3204–3213.
62. Douglas MW, Diefenbach RJ, Homa FL, *et al*. Herpes simplex virus type 1 capsid protein VP26 interacts with dynein light chains RP3 and Tctex1 and plays a role in retrograde cellular transport. *J Biol Chem* 2004; **279**: 28522–28530.
63. Desai P, DeLuca NA, Person S. Herpes simplex virus type 1 VP26 is not essential for replication in cell culture but influences production of infectious virus in the nervous system of infected mice. *Virology* 1998; **247**: 115–124.
64. Kramer MF, Chen SH, Knipe DM, *et al*. Accumulation of viral transcripts and DNA during establishment of latency by herpes simplex virus. *J Virol* 1998; **72**: 1177–1185.
65. Antinone SE, Shubeita GT, Coller KE, *et al*. The Herpesvirus capsid surface protein, VP26, and the majority of the tegument proteins are dispensable

DOI: 10.1002/rmv

for capsid transport toward the nucleus. *J Virol* 2006; **80**: 5494–5498.
66. Dohner K, Radtke K, Schmidt S, *et al*. Eclipse phase of herpes simplex virus type 1 infection: efficient dynein-mediated capsid transport without the small capsid protein VP26. *J Virol* 2006; **80**: 8211–8224.
67. Tomishima MJ, Smith GA, Enquist LW. Sorting and transport of alpha herpesviruses in axons. *Traffic* 2001; **2**: 429–436.
68. Granzow G, Klupp BG, Mettenleiter TC. Entry of pseudorabies virus: an immunogold-labeling study. *J Virol* 2005; **79**: 3200–3205.
69. Luxton GW, Haverlock S, Coller KE, *et al*. Targeting of herpesvirus capsid transport in axons is coupled to association with specific sets of tegument proteins. *Proc Natl Acad Sci USA* 2005; **102**: 5832–5837.
70. Wolfstein A, Nagel CH, Radtke K, *et al*. The inner tegument promotes herpes simplex virus capsid motility along microtubules in vitro. *Traffic* 2006; **7**: 227–237.
71. Farnsworth A, Goldsmith KA, Johnson DC. Herpes simplex virus glycoproteins gD and gE/gI serve essential but redundant functions during acquisition of the virion envelope in the cytoplasm. *J Virol* 2003; **77**: 481–8494.
72. Kopp M, Granzow H, Fuchs W, *et al*. The pseudorabies virus UL11 protein is a virion component involved in secondary envelopment in the cytoplasm. *J Virol* 2003; **77**: 5339–5351.
73. Wozniak MJ, Milner R, Allan V. N-terminal kinesins: many and various. *Traffic* 2004; **5**: 400–410.
74. Hirokawa N, Takemura R. Kinesin superfamily proteins and their various functions and dynamics. *Exp Cell Res* 2004; **301**: 50–59.
75. Hirokawa N, Takemura R. Molecular motors and mechanisms of directional transport in neurons. *Nat Rev Neurosci* 2005; **6**: 201–214.
76. Miki H, Okada Y, Hirokawa N. Analysis of the kinesin superfamily: insights into structure and function. *Trends Cell Biol* 2005; **15**: 467–476.
77. Miki H, Setou M, Kaneshiro K, *et al*. All kinesin superfamily protein, KIF, genes in mouse and human. *Proc Natl Acad Sci USA* 2001; **98**: 7004–7011.
78. Lawrence CJ, Dawe RK, Christie KR, *et al*. A standardized kinesin nomenclature. *J Cell Biol* 2004; **167**: 19–22.
79. Vale RD, Reese TS, Sheetz MP. Identification of a novel force-generating protein, kinesin, involved in microtubule-based motility. *Cell* 1985; **42**: 39–50.
80. Hirokawa N, Pfister KK, Yorifuji H, *et al*. Submolecular domains of bovine brain kinesin identified by electron microscopy and monoclonal antibody decoration. *Cell* 1989; **56**: 867–878.
81. Adio S, Reth J, Bathe F, *et al*. Review: regulation mechanisms of Kinesin-1. *J Muscle Res Cell Motil* 2006; **27**: 153–160.
82. Kirchner J, Seiler S, Fuchs S, *et al*. Functional anatomy of the kinesin molecule in vivo. *EMBO J* 1999; **18**: 4404–4413.
83. Skoufias DA, Cole DG, Wedaman KP, *et al*. The carboxyl-terminal domain of kinesin heavy chain is important for membrane binding. *J Biol Chem* 1994; **269**: 1477–1485.
84. Mettenleiter TC. Herpesvirus assembly and egress. *J Virol* 2002; **76**: 1537–1547.
85. Luxton GW, Lee JI, Haverlock-Moyns S, *et al*. The pseudorabies virus VP1/2 tegument protein is required for intracellular capsid transport. *J Virol* 2006; **80**: 201–209.
86. Ogawa-Goto K, Irie S, Omori A, *et al*. An endoplasmic reticulum protein, p180, is highly expressed in human cytomegalovirus-permissive cells and interacts with the tegument protein encoded by UL48. *J Virol* 2002; **76**: 2350–2362.
87. Diefenbach RJ, Diefenbach E, Douglas MW, *et al*. The ribosome receptor, p180, interacts with kinesin heavy chain, KIF5B. *Biochem Biophys Res Commun* 2004; **319**: 987–992.
88. Braun N, Schikorski T, Zimmermann H. Cytoplasmic segregation and cytoskeletal organization in the electric catfish giant electromotoneuron with special reference to the axon hillock region. *Neuroscience* 1993; **52**: 745–756.
89. Miranda-Saksena M, Boadle RA, Armati P, *et al*. In rat dorsal root ganglion neurons, herpes simplex virus type 1 tegument forms in the cytoplasm of the cell body. *J Virol* 2002; **76**: 9934–9951.
90. Saksena MM, Wakisaka H, Tijono B, *et al*. Herpes simplex virus type 1 accumulation, envelopment, and exit in growth cones and varicosities in mid-distal regions of axons. *J Virol* 2006; **80**: 3592–3606.
91. Penfold ME, Armati P, Cunningham AL. Axonal transport of herpes simplex virions to epidermal cells: evidence for a specialized mode of virus transport and assembly. *Proc Natl Acad Sci USA* 1994; **91**: 6529–6533.
92. Holland DJ, Miranda-Saksena M, Boadle RA, *et al*. Anterograde transport of herpes simplex virus proteins in axons of peripheral human fetal neurons: an immunoelectron microscopy study. *J Virol* 1999; **73**: 8503–8511.
93. Miranda-Saksena M, Armati P, Boadle RA, *et al*. Anterograde transport of herpes simplex virus type 1 in cultured, dissociated human and rat dorsal root ganglion neurons. *J Virol* 2000; **74**: 1827–1839.
94. Ohara PT, Chin MS, LaVail JH. The spread of herpes simplex virus type 1 from trigeminal

DOI: 10.1002/rmv

neurons to the murine cornea: an immunoelectron microscopy study. *J Virol* 2000; **74**: 4776–4786.
95. Ohara PT, Tauscher AN, LaVail JH. Two paths for dissemination of Herpes simplex virus from infected trigeminal ganglion to the murine cornea. *Brain Res* 2001; **899**: 260–263.
96. Tomishima MJ, Enquist LW. A conserved alpha-herpesvirus protein necessary for axonal localization of viral membrane proteins. *J Cell Biol* 2001; **154**: 741–752.
97. Enquist LW, Tomishima MJ, Gross S, *et al*. Directional spread of an alpha-herpesvirus in the nervous system. *Vet Microbiol* 2002; **86**: 5–16.
98. Tomishima MJ, Enquist LW. In vivo egress of an alphaherpesvirus from axons. *J Virol* 2002; **76**: 8310–8317.
99. LaVail JH, Tauscher AN, Aghaian E, *et al*. Axonal transport and sorting of herpes simplex virus components in a mature mouse visual system. *J Virol* 2003; **77**: 6117–6126.
100. Lavail JH, Tauscher AN, Hicks JW, *et al*. Genetic and molecular in vivo analysis of herpes simplex virus assembly in murine visual system neurons. *J Virol* 2005; **79**: 11142–11150.
101. LaVail JH, Tauscher AN, Sucher A, *et al*. Viral regulation of the long distance axonal transport of herpes simplex virus nucleocapsid. *Neuroscience* 2007; **146**: 974–985.
102. Snyder A, Bruun B, Browne HM, *et al*. A herpes simplex virus gD-YFP fusion glycoprotein is transported separately from viral capsids in neuronal axons. *J Virol* 2007; **81**: 8337–8340.
103. Ch'ng TH, Enquist LW. Neuron-to-cell spread of pseudorabies virus in a compartmented neuronal culture system. *J Virol* 2005; **79**: 10875–10889.
104. del Rio T, Ch'ng TH, Flood EA, *et al*. Heterogeneity of a fluorescent tegument component in single pseudorabies virus virions and enveloped axonal assemblies. *J Virol* 2005; **79**: 3903–3919.
105. Smith GA, Gross SP, Enquist LW. Herpesviruses use bidirectional fast-axonal transport to spread in sensory neurons. *Proc Natl Acad Sci USA* 2001; **98**: 3466–3470.
106. De Regge N, Nauwynck HJ, Geenen K, *et al*. Alpha-herpesvirus glycoprotein D interaction with sensory neurons triggers formation of varicosities that serve as virus exit sites. *J Cell Biol* 2006; **174**: 267–275.
107. Antinone SE, Smith GA. Two modes of herpesvirus trafficking in neurons: membrane acquisition directs motion. *J Virol* 2006; **80**: 11235–11240.
108. Klupp BG, Fuchs W, Granzow H, *et al*. Pseudorabies virus UL36 tegument protein physically interacts with the UL37 protein. *J Virol* 2002; **76**: 3065–3071.
109. Hill TJ, Field HJ. The interaction of herpes simplex virus with cultures of peripheral nervous tissue: an electron microscopic study. *J Gen Virol* 1973; **21**: 123–133.
110. Kristensson K, Ghetti B, Wisniewski HM. Study on the propagation of Herpes simplex virus (type 2) into the brain after intraocular injection. *Brain Res* 1974; **69**: 189–201.
111. Cook ML, Stevens JG. Pathogenesis of herpetic neuritis and ganglionitis in mice: evidence for intra-axonal transport of infection. *Infect Immun* 1973; **7**: 272–288.
112. Yamamoto T, Otani S, Shiraki H. Ultrastructure of herpes simplex virus infection of the nervous system of mice. *Acta Neuropathol* 1973; **26**: 285–299.
113. Lycke E, Kristensson K, Svennerholm B, *et al*. Uptake and transport of herpes simplex virus in neurites of rat dorsal root ganglia cells in culture. *J Gen Virol* 1984; **65**: 55–64.
114. Card JP, Rinaman L, Lynn RB, *et al*. Pseudorabies virus infection of the rat central nervous system: ultrastructural characterization of viral replication, transport, and pathogenesis. *J Neurosci* 1993; **13**: 2515–2539.
115. LaVail JH, Topp KS, Giblin PA, *et al*. Factors that contribute to the transneuronal spread of herpes simplex virus. *J Neurosci Res* 1997; **49**: 485–496.
116. Hill TJ, Field HJ, Roome AP. Intra-axonal location of herpes simplex virus particles. *J Gen Virol* 1972; **15**: 233–235.
117. Field HJ, Hill TJ. The pathogenesis of pseudorabies in mice following peripheral inoculation. *J Gen Virol* 1974; **23**: 145–157.
118. Diefenbach RJ, Miranda-Saksena M, Diefenbach E, *et al*. Herpes simplex virus tegument protein US11 interacts with conventional kinesin heavy chain. *J Virol* 2002; **76**: 3282–3291.
119. Benboudjema L, Mulvey M, Gao Y, *et al*. Association of the herpes simplex virus type 1 Us11 gene product with the cellular kinesin light-chain-related protein PAT1 results in the redistribution of both polypeptides. *J Virol* 2003; **77**: 9192–9203.
120. Longnecker R, Roizman B. Clustering of genes dispensable for growth in culture in the S component of the HSV-1 genome. *Science* 1987; **236**: 573–576.
121. Satpute-Krishnan P, DeGiorgis JA, Bearer EL. Fast anterograde transport of herpes simplex virus: role for the amyloid precursor protein of alzheimer's disease. *Aging Cell* 2003; **2**: 305–318.
122. Koshizuka T, Kawaguchi Y, Nishiyama Y. Herpes simplex virus type 2 membrane protein UL56 associates with the kinesin motor protein KIF1A. *J Gen Virol* 2005; **86**: 527–533.
123. Ch'ng TH, Enquist LW. Efficient axonal localization of alphaherpesvirus structural proteins in cul-

DOI: 10.1002/rmv

tured sympathetic neurons requires viral glycoprotein E. *J Virol* 2005; **79**: 8835–8846.
124. Wang F, Tang W, McGraw HM, *et al*. Herpes simplex virus type 1 glycoprotein e is required for axonal localization of capsid, tegument, and membrane glycoproteins. *J Virol* 2005; **79**: 13362–13372.
125. Feierbach B, Bisher M, Goodhouse J, *et al*. In vitro analysis of transneuronal spread of an alphaherpesvirus infection in peripheral nervous system neurons. *J Virol* 2007; **81**: 6846–6857.
126. Kelkar SA, Pfister KK, Crystal RG, *et al*. Cytoplasmic Dynein mediates adenovirus binding to microtubules. *J Virol* 2004; **78**: 10122–10132.
127. Suomalainen M, Nakano MY, Keller S, *et al*. Microtubule-dependent plus- and minus end-directed motilities are competing processes for nuclear targeting of adenovirus. *J Cell Biol* 1999; **144**: 657–672.
128. Ligon LA, Tokito M, Finklestein JM, *et al*. A direct interaction between cytoplasmic dynein and kinesin I may coordinate motor activity. *J Biol Chem* 2004; **279**: 19201–19208.
129. Smith GA, Pomeranz L, Gross SP, *et al*. Local modulation of plus-end transport targets herpesvirus entry and egress in sensory axons. *Proc Natl Acad Sci USA* 2004; **101**: 16034–16039.
130. Welte MA. Bidirectional transport along microtubules. *Curr Biol* 2004; **14**: R525–R537.

DOI: 10.1002/rmv