Microreview

Viral interactions with the cytoskeleton: a hitchhiker’s guide to the cell

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Summary

The actin and microtubule cytoskeleton play important roles in the life cycle of every virus. During attachment, internalization, endocytosis, nuclear targeting, transcription, replication, transport of progeny subviral particles, assembly, exocytosis, or cell-to-cell spread, viruses make use of different cellular cues and signals to enlist the cytoskeleton for their mission. Viruses induce rearrangements of cytoskeletal filaments so that they can utilize them as tracks or shove them aside when they represent barriers. Viral particles recruit molecular motors in order to hitchhike rides to different subcellular sites which provide the proper molecular environment for uncoating, replicating and packaging viral genomes. Interactions between subviral components and cytoskeletal tracks also help to orchestrate virus assembly, release and efficient cell-to-cell spread. There is probably not a single virus that does not use cytoskeletal and motor functions in its life cycle. Being well informed intracellular passengers, viruses provide us with unique tools to decipher how a particular cargo recruits one or several motors, how these are activated or tuned down depending on transport needs, and how cargoes switch from actin tracks to microtubules to nuclear pores and back.

A virus’ journey through the cell

Viruses use the host actin and microtubule transport systems and their motors (Fig. 1) for several steps during their life cycle, which is truly a journey through the entire cell (Cudmore et al., 1997; Sodeik, 2000; Ploubidou and Way, 2001; Smith and Enquist, 2002; Döhner and Sodeik, 2004; Smith and Helenius, 2004). The voyage starts at the cell periphery with virus attachment to the plasma membrane and internalization, after which viruses or subviral particles travel further towards the cell centre, often riding inside endocytic organelles (Fig. 2; Sieczkarski and Whittaker, 2004; Smith and Helenius, 2004). To reach the nucleoplasm or the cytosol, where nucleotides required for replication are available, all viruses translocate their genome across the plasma or an endosomal membrane (Whittaker, 2003; Greber and Fornerod, 2004). Newly synthesized viral proteins and genomes then move to sites of virus assembly, which is initiated in the nucleus or the cytosol, and frequently proceeds on intracellular membranes or the plasma membrane. Several viruses have evolved specific egress pathways for transport back to the plasma membrane, often using the cell’s secretory pathway via the endoplasmic reticulum (ER), the Golgi apparatus, and even transport vesicles (Fig. 3; Garoff et al., 1998; Johnson and Huber, 2002; Pelchen-Matthews et al., 2004).

The presence of many organelles and the cytoskeleton, particularly the actin filaments, and molecular crowding caused by high protein concentration restrict free diffusion of molecules larger than 500 kDa in the cytoplasm compared with dilute solutions (Luby-Phelps, 2000; Sodeik, 2000; Verkman, 2002; Dauty and Verkman, 2005). Therefore, viruses and also host organelles require active mechanisms for directed transport. In the cell periphery and possibly in the nucleus, transport is mediated by the actin system, either by newly polymerized actin filaments pushing a particle, or by myosins moving along actin filaments. The motor proteins cytoplasmic dynein and kinesin catalyse transport along microtubules, thus bridging the gap between the periphery and the cell centre. In this review, we discuss recent discoveries on the manifold ways how viruses hijack the intracellular shipping machinery for their own transport, with particular emphasis on processes involving actin and microtubules. Interactions of viruses with the third component of the cytoskeleton, the intermediate filaments, are discussed elsewhere (Lake et al., 2003; Döhner and Sodeik, 2004).
Numerous viral proteins interact with actin-binding proteins or directly with actin (Cudmore et al., 1997; Ploubidou and Way, 2001; Smith and Enquist, 2002; Döhner and Sodeik, 2004; Smith and Helenius, 2004). The infectious particles of many viruses, e.g., retroviruses, picornaviruses and herpesviruses, even contain actin (Cudmore et al., 1997; Newman and Brown, 1997; Grünwald et al., 2003; Johansen et al., 2004; Varnum et al., 2004; Cantin et al., 2005; del Rio et al., 2005; Zhu et al., 2005). Actin filaments of 7–9 nm thickness (Fig. 1) are built by head-to-tail assembly of polar monomeric actin. Therefore, the resulting filaments are also polar and characterized by a fast growing plus-end and a slow growing minus-end, whose dynamics are controlled by a plethora of actin-binding proteins (Pollard and Borisy, 2003; Winder and Ayscough, 2005). Actin filaments assemble further into higher-order structures, such as linear bundles, two-dimensional networks, or three-dimensional gels.

While actin is present throughout the entire cytosol, its concentration is highest directly underneath the plasma membrane. This actin cortex, a three-dimensional meshwork linked to the plasma membrane via integral and peripheral membrane proteins, enables the cell to move by stretching out filopodia and lamellipodia, and provides mechanical strength, in cell culture mainly via stress fibres (Pollard and Borisy, 2003). Therefore, actin is the key player in cell migration. However, it also mediates various intracellular transport processes. These dynamics are either based on controlled actin polymerization or on myosins which upon ATP hydrolysis translocate along actin filaments.

Myosin motors are made of one or two heavy chains (Fig. 1) and one or more light chains (Kieke and Titus, 2003; Krendel and Mooseker, 2005). A heavy chain consists of a conserved N-terminal motor domain which binds...
Fig. 2. Viral entry into the cell – getting in and travelling downtown. The virus depicted here and in Fig. 3 is a hypothetical model. Virus interactions with the cytoskeleton differ between virus families, and no virus is currently known to use all of the mechanisms depicted here, but might employ different strategies. The hypothetical virus binds to the plasma membrane and surfs along filopodia (1) towards an area of high endocytic activity (2), where it is internalized by endocytosis (3). Alternatively, the virus can fuse with the plasma membrane (4). After traversing the actin cortex either inside endocytic vesicles or by itself, free viruses or viruses inside vesicles are transported by dynein/dynactin along microtubules towards the MTOC (5). From the MTOC, viruses are transported towards the nucleus, possibly aided by the nuclear import/export machinery (6). Upon binding to the nuclear pore, the virus releases its genome for replication (7).

actin filaments and hydrolyses ATP, a converter domain that generates the force required for movement, a neck domain that binds the light chains, and a divergent C-terminal globular tail implicated in cargo binding. Based on sequence homology in the heavy chain, 20 different classes of myosins have been identified. However, not all classes are present in a single organism, e.g. humans have 12 classes of myosin genes. Myosins move unidirectionally along actin filaments. In general, actin filaments point with their plus-ends towards the plasma membrane. Therefore, plus-end directed myosins, like myosin I or V, will carry a cargo to the cell periphery, or if bound to the plasma membrane, translocate actin filaments towards the cell centre. Minus-end directed myosins, such as myosin VI and possibly myosin IXb, move in the opposite direction (Kieke and Titus, 2003; Krendel and Mooseker, 2005).

Microtubules, dynein and kinesin motors

For long distances, e.g. as in axons, dyneins and kinesins propel cellular cargo along microtubules. As experienced cell travellers, viruses also use these highways, either as hitchhikers inside cellular vesicles or...
organelles, or as cytosolic viral complexes that hijack microtubule motors directly (Smith and Enquist, 2002; Döhner et al., 2005). Microtubules are long, hollow cylinders made of tubulin. Similarly to actin, tubulin is also occasionally packaged into virions (Johannsen et al., 2004; Varnum et al., 2004; Cantin et al., 2005). As the tubulin heterodimers all assemble in the same orientation, microtubules have an intrinsic polarity with the plus-ends having a higher propensity to polymerize or depolymerize than the minus-ends. Several microtubule-associated proteins bind preferentially to one or the other end, and thereby regulate the dynamic behaviour of microtubules (Dammernmann et al., 2003; Akhmanova and Hoo-
Each microtubule motor walks only in one direction, and cellular or viral cargoes thus use specific motors to reach different regions of a cell (Fig. 1). While cytoplasmic dynein catalyses most minus-end directed transport, members of the kinesin superfamily are responsible for plus-end directed movement. However, some kinesins, such as KIFC2, also translocate in a minus-end direction (Ovechkina and Wordeman, 2003). Cytoplasmic dynein contains two heavy chains (DHC) which bind to microtubules and hydrolyse ATP. In addition, there are up to six light chains belonging to three protein families (LC8/PIN, tctex, and LC7/roadblock), two light intermediate chains (DIC), and two intermediate chains (DIC), all of which have been implicated in cargo binding (Susalka and Pfister, 2000; Vallee et al., 2004). Cytoplasmic dynein is associated with dynactin, which is required for most, if not all, dynein-driven processes (Schroer, 2004). Dynactin enhances dynein processivity and is involved in cargo binding. The kinesins come in different assemblies (Vale, 2003; Hirokawa and Takemura, 2005; Yildiz and Selvin, 2005): conventional kinesin-1 contains two light chains and two motor subunits, heterotrimeric kinesin-2 consists of two different subunits which both have motor activity and a third subunit called kinesin associated protein, while kinesin-3 and kinesin-4 are composed of one or two motor subunits respectively. The large motor subunits bind microtubules and ATP and can be involved in cargo binding, whereas the non-motor subunits are responsible for transport regulation and cargo binding. Recent findings show that dynactin is also involved in kinesin-2-mediated transport (Deacon et al., 2003).

**Virus binding and surging**

The first step of a virus’ journey into a cell is binding to viral receptors displayed on the plasma membrane (Dimlitrov, 2004). While some receptors just mediate attachment, virus binding to others induces conformational changes in the receptor, in the virion, or in both, and these conformational changes trigger virus internalization, e.g. by endocytosis or fusion with or penetration of the plasma membrane (Sieczkarski and Whittaker, 2004; Smith and Helenius, 2004).

Even before having entered a cell, viruses already interact with the actin cytoskeleton in a number of ways. Several retroviruses and particles pseudotyped with the envelope protein of vesicular stomatitis virus surf along filopodia and retraction fibres towards the cell body [Fig. 2(1)] prior to either being internalized at the filopodium base or moving further along the plasma membrane to a clathrin-containing region respectively (Lehmann et al., 2005). This cell surfing requires dynamic actin, myosin II and cognate virus-receptor interactions, and seems to mimic the transport of ligand-bound, activated growth factor receptors prior to their endocytosis at the filopodium base (Lidke et al., 2005). During surfing, the virus is probably coupled through the cytosolic domain of its receptor to an actin filament inside the filopodium. This filament may then be pulled towards the base of the filopodium by myosin II which itself is anchored to the actin cortex (Jay, 2000). The actin filaments continuously polymerize at the tip of the filopodium to maintain its length. This surfing may provide transportation to a region of the plasma membrane where virus internalization is possible.

In other regions of the plasma membrane, the actin cortex restricts diffusion of virions bound to their receptors. After attachment [Fig. 2(2)], surface-bound polynavirus diffuses freely for about 5–10 s before their movement is confined in an actin-dependent manner (Ewers et al., 2005). Similarly, simian virus 40 (SV40) virions move laterally in a random fashion until trapped in immobile spots corresponding to caveolae (Pelkmans et al., 2002). When filamentous actin is depolymerized, SV40 remains mobile even after reaching caveolae. Likewise, actin depolymerization increases the lateral movement of clathrin-coated pits in the plane of the plasma membrane (Gaidarov et al., 1999; Yarar et al., 2005), suggesting that cortical actin filaments may function as a scaffold that orchestrates the lateral mobility of both caveolae and clathrin-coated pits.

**Virus internalization – getting in**

Having bound to the cell surface, the virus faces two obstacles: the plasma membrane and the actin cortex (Marsh and Bron, 1997; Medalia et al., 2002). Several viruses take one of the various endocytosis routes to get a free ride into the cytoplasm, e.g. Semliki Forest virus, influenza virus and adenovirus enter via clathrin-coated pits, whereas SV40, other polyomaviruses and some picornaviruses are internalized into caveolae [Fig. 2(3); Sieczkarski and Whittaker, 2004; Smith and Helenius, 2004]. In contrast, HIV, measles virus or herpes simplex virus can fuse directly with the plasma membrane [Fig. 2(4)], and then have to find their way through the actin cortex on their own (Sodeik, 2000; Döhner and Sodeik, 2004; Smith and Helenius, 2004).

In uninfected cells, endocytosis and exocytosis are accompanied by local actin dynamics which may open channels for vesicles to pass through, or provide tracks to move on (Apodaca, 2001; Giner et al., 2005). Therefore, the actin cortex is no barrier for endocytosis, but participates as a scaffold, modulates invagination and scission, and may even contribute actively to vesicle transport (Apodaca, 2001; Yarar et al., 2005). Myosin VI colocalizes with endocytic vesicles and may catalyse their transport from the plasma membrane towards an early endosome (Buss et al., 2004), and possibly also that of cytosolic...
virus towards the cell interior. Entry of adenovirus type 2 and a retrovirus pseudotyped with Ebola virus glycoprotein is blocked when actin filaments are stabilized with jasplakinolide or destabilized by cytochalasin D, indicating a requirement of dynamic actin filaments for their uptake (Nakano et al., 2000; Meier et al., 2002; Yonezawa et al., 2005). Similarly, infection with HIV was less effective after inhibiting the Arp2/3 complex, which promotes actin polymerization (Komano et al., 2004). Viruses can interfere with cellular signalling pathways and thus induce actin rearrangements supporting infection. SV40 induced tyrosine phosphorylation causes depolymerization of actin stress fibres and a concomitant recruitment of actin patches and tails to caveolae containing SV40 virions (Pelkmans et al., 2002). SV40 and caveolin localize to the same end of these dynamic actin tails. In addition, the inhibition of PI(4,5)-kinase and Cdc42 pathways destabilizes the actin cortex and thus increases the efficiency of SV40 infection (Pelkmans et al., 2005).

Having maneuvered through the actin cortex either inside vesicles or by themselves, viruses, like endosomes, seem to switch from actin to microtubule tracks to get to the cell centre. This may be accomplished by binding myosin and dynein simultaneously or sequentially, thus enabling the virus to step from one track to the next once it is in close proximity to the microtubules.

### Nuclear targeting – travelling downtown avoiding bad neighbourhoods

After internalization, the virus proceeds to its replication site, either the cytosol or the nucleoplasm, and uncoats its genome. A number of viruses have been shown to require microtubules and dynein for efficient nuclear targeting [Fig. 2(5)], either for transport of cytosolic viral particles or virions inside endosomes (Smith and Enquist, 2001; Suikkanen et al., 2003). Murine polyomavirus is transported along microtubules as cytosolic cargo after leaving endocytic vesicles, and the transport of adeno-associated virus also depends on microtubules (Seisenberger et al., 2001; Sanjuan et al., 2003). A retrovirus pseudotyped with Ebola virus glycoprotein and influenza virus are transported along microtubules inside vesicles until reaching the MTOC (Lakadamyali et al., 2003; Yonezawa et al., 2005). Species C adenoviruses and canine parvovirus are also first transported inside vesicles, but leave these either early in the cell periphery or late close to the MTOC, respectively, to recruit dynein in order to head towards the nucleus (Suomalainen et al., 1999; 2001; Leopold et al., 2000; Suikkanen et al., 2003). Human foamy virus, herpes simplex virus, and HIV can use many cell types by fusion at the plasma membrane, and the incoming cytosolic capsids or preintegration complexes also use dynein and dynactin (Sodeik et al., 1997; Döhner et al., 2002; McDonald et al., 2002; Petit et al., 2003).

Kaposi’s sarcoma-associated herpesvirus glycoprotein B binds to α3β1 integrin at the cell surface, thus activating a signalling cascade, which, via focal adhesion kinase, Src and PI3-kinase, stimulates the small GTPase Rho to stabilize microtubules. After internalization, the virus exploits dynein and dynactin to travel inside endosomes along these stable microtubules (Sharma-Walia et al., 2004; Narantset et al., 2005). Adenoviruses stimulate dynein mediated transport during entry by transiently activating protein kinase A and p38/MAPK pathways (Suomalainen et al., 2001). Finally, herpesviruses can regulate the direction of their bidirectional transport in axons by modulating the amount of plus-end directed transport. This results either in nuclear targeting when minus-end directed transport is more frequent and more processive than plus-end directed transport, or in transport towards the plasma membrane when plus-end directed transport predominates (Smith et al., 2001; 2004).

Viral components may bind directly to dynein by binding dynein intermediate chain (DIC), and yet more interactions have been identified between viral proteins and dynein light chains (DLCs) of the LC8 and tctex families (Döhner et al., 2005). However, in many cases the putative cargo-binding to the LC8 dimer would compete with its binding to DIC, which attaches the LC8 dimer to the dynein complex. Thus, to be able to work as a virus receptor, one of the two LC8 molecules in the dynein complex would need to disengage from DIC to bind to the viral cargo, while the other LC8 molecule would remain bound to the other DIC, resulting in an asymmetric complex. As LC8 is also part of other protein complexes, viral proteins may also bind to LC8 for other functions besides recruiting dynein (Vallee and Hook, 2003; Vallee et al., 2004; Wu et al., 2005).

Members of the tctex family have a cargo-binding region that is distinct from their DIC-binding domain, and thus could more easily attach a viral cargo to dynein (Vallee et al., 2004; Wu et al., 2005). However, the tctex binding motif of some putative cargoes are similar to the tctex binding motif of DIC (Sugai et al., 2003). The outer capsid protein VP26 of herpes simplex virus interacts with DLCs of the tctex family (Douglas et al., 2004). A VP26 deletion mutant that is still able to use retrograde axonal transport to access the trigeminal ganglion might enter neurons via endocytosis and travel within vesicles to the neuronal cell nucleus (Desai et al., 1998). However, capsids of herpes simplex virus bind dynein in vitro with similar efficiencies in the absence and presence of VP26 (Wolfstein et al., 2006). Moreover, using GFPVP26-tagged capsids, Wolfstein and colleagues showed that components of the inner tegument, a layer between the capsid and the herpesvirus envelope, are
required for efficient microtubule transport. In contrast, capsids purified from infected nuclei which contain almost no tegument but expose VP26 on their surface are unable to recruit dynein and dynactin, and are immobile in the in vitro motility assay (Wolfstein et al., 2006). These data suggest that in addition to VP26, herpes simplex virus tegument proteins are required to enlist dynein.

Instead of binding to motor subunits, viruses could also hook onto host adaptors and indirectly employ dynein. Dynactin, a 20 S complex consisting of 10 different proteins, connects many cellular cargoes to dynein (Schoer, 2004). Disruption of dynactin impedes transport of herpes simplex virus, adenovirus, vaccinia virus and African swine fever virus towards the MTOC (Suomalainen et al., 1999; Ploubidou et al., 2000; Alonso et al., 2001; Döhner et al., 2002). Surprisingly, other potential adaptors are nuclear import factors such as the importins (Pemberton and Paschal, 2005). After axonal injury, a retrograde signalling complex containing importin β recruits dynein via importin α and is then transported to the cell body to promote repair (Hanz et al., 2003). Other cytosolic proteins bearing nuclear localization signals also bind dynein and importin β, and are conveyed towards the nucleus along microtubules (Mesika et al., 2005; Salman et al., 2005). It is tempting to speculate that some of the several viral proteins binding to importins (Whittaker, 2003; Greber and Fornerod, 2004) may also enlist dynein via this link. Another viral dynein anchor may be the genomic RNA, or mRNAs packaged, e.g., in the tegument of herpesviruses (Bresnahan and Shenk, 2000; Sciortino et al., 2001; Bechtel et al., 2005). The cytosolic transport and subcellular localization of some host mRNAs is mediated by microtubule motors (Kindler et al., 2005).

Dynein gets only as far as the MTOC [Fig. 2(5)], which in many, but not all cells is located close to the nucleus. Among the physiological cargo that dynein transports are protein aggregates that are packed into aggresomes at the MTOC (Garcia-Mata et al., 2002). These aggresomes, which might be considered as cell garbage, either recruit chaperones for refolding or proteasomes for protein degradation, or they are eliminated by autophagy that terminates in lysosomal degradation (Kopito, 2000; Kirkegaard et al., 2004). If the cell treats a viral capsid as an aggresome, the area around the MTOC would be a dangerous neighbourhood. This idea is consistent with experiments in which proteasome inhibitors stimulated the efficiency of adeno-associated virus and HIV infection (Deroo and Archer, 2002; Yan et al., 2002; Wei et al., 2005). However, other viruses such as influenza virus, minute virus of mice, Sendai virus, vesicular stomatitis virus or mouse hepatitis virus require proteasome activity to initiate infection (Ros et al., 2002; Khor et al., 2003; Watanebe et al., 2005; Yu and Lai, 2005).

The connection between dynein and nuclear import/export factors mentioned above may bridge the gap from the MTOC to the nuclear pore, which is a part of the viral journey not well understood [Fig. 2(6)]. A recent study shows that the nuclear export factor CRM1 is required for adenovirus transport from the MTOC to the nucleus (Strunze et al., 2005). In addition, kinesins may catalyse the plus-end directed microtubule transport from the MTOC to the nucleus. Interestingly, CRM1 interacts with the protein RanBP2 (Singh et al., 1999; Bernad et al., 2004), which in turn can bind kinesin-1 (Cai et al., 2001). Whatever the transport mechanism for this part of the itinerary may be, at the nuclear pore or at replication sites in the cytosol viruses disassemble, uncoat their genome, and release it into the nucleoplasm or cytosol, where viral replication takes place [Fig. 2(7); Whittaker, 2003; Greber and Fornerod, 2004].

Genome replication – the purpose of the trip

The purpose of a virus entering a cell is to generate progeny. After arrival in the cytosol or the nucleoplasm, viral genomes are replicated and packaged by newly synthesized viral proteins [Fig. 3(1)]. Viral replication often occurs in specific regions of the nucleus or the cytosol, and the host’s replication apparatus is modified to suit a virus’ needs, for example, by actin tethering replication enzymes or transporting them to a viral factory. Nuclear actin has been implicated in typical nuclear activities such as transcription, splicing, mRNA export, and chromatin remodelling, and may contribute to a nucleoskeleton and the nuclear lamina (Bettinger et al., 2004; Pederson and Aebi, 2005). The motor myosin I and other actin-binding proteins are involved in nuclear transcription (Philiponeno et al., 2004), and a nuclear actin-myosin system may mediate the movement of the ND10 nuclear bodies that are modified by many viruses replicating in the nucleus (see Muratani et al., 2002; Simpson-Holley et al., 2005; Everett, 2006).

During cytosolic replication, viruses may also interact with the cytoskeleton. Vaccinia virus mRNA colocalizes with microtubules, which are required for viral gene expression (Mallardo et al., 2001; Schramm and Locker, 2005). The RNA genome of human parainfluenza virus type 3 is replicated and transcribed in association with ribonucleoproteins bound to actin filaments, and depolymerization of actin inhibits viral RNA synthesis (Gupta et al., 1998). Newly synthesized mRNAs of influenza virus are associated with the viral nucleocapsid protein which can bind to actin filaments and whose nuclear import and export are carefully regulated to retain the ribonucleoprotein complex in the cytosol for efficient protein translation (Portela and Digard, 2002). Microtubules and actin can not only anchor the transcription apparatus, but also
sequester and thus inactivate regulatory factors. Human respiratory syncytial virus depends on the presence of actin for in vitro transcription, which is further stimulated by the actin-binding protein profilin (Burke et al., 2000). Sendai virus transcription is enhanced by tubulin binding to the matrix protein, a negative regulator of viral transcription (Ogino et al., 2003). Thus actin and tubulin perform additional regulatory functions different from transport.

**Virus assembly – packing up**

After genome replication and protein synthesis, the subviral components are transported to the site where virus assembly commences. In the case of cytosolic replication, new genomes can bind directly to proteins as they emerge from ribosomes. After nuclear replication, subviral particles containing the genome are either exported through the nuclear pore as shown for influenza virus or HIV, or the genome is packaged into a nuclear capsid, as is the case for herpesviruses, adenovirus and baculovirus (Whittaker and Helenius, 1998; Whittaker, 2003; Döhner and Sodeik, 2004). A recent study suggests that progeny nuclear capsids of herpes simplex virus may be actively transported to the nuclear membrane for primary budding, a process which is abolished by drug induced depolymerization of actin filaments and by inhibiting myosin activity [Fig. 3(2); Forest et al., 2005]. The export of HIV genomic RNA to the cytosol also seems to require nuclear actin (Kimura et al., 2000; Hofmann et al., 2001). HIV RNAs contain Rev-responsive elements and colocalize with the nuclear export factor CRM1 and the GTPase Ran on nuclear actin bundles, and RNA export is diminished after treatment with the actin depolymerizing drug latrunculin B or the injection of antibodies against actin. After export into the cytosol, the viral gag protein is synthesized, which then tethers viral RNA to actin filaments close to the MTOC (Poole et al., 2005), thus initiating the next step of the viral journey.

Viral membrane and glycoproteins take a different route than cytosolic viral complexes. They are synthesized by ribosomes of the ER and processed while being transported through the ER towards the Golgi apparatus located around the MTOC [Fig. 3(4)], and further to their final destination. Viruses have different strategies for final assembly, which may take place in the cytosol, the nucleus or by budding into different membranes such as the ER, the Golgi apparatus, the trans-Golgi network, the plasma membrane and even the endosomes [Fig. 3(5); Smith and Enquist, 2002; Mettenleiter, 2004; Pelchen-Matthews et al., 2004].

Depending on the subcellular localization of the budding organelle, cytosolic viral particles and viral components in association with membranes are transported by minus- or plus-end directed microtubule motors to the site of virus assembly. Vaccinia virus, African swine fever virus and gag precursor proteins of Mason-Pfizer monkey virus proceed towards the MTOC [Fig. 3(3)], using dynein again for this later stage of their journey through the cell (Plou-bidou et al., 2000; Sanderson et al., 2000; Alonso et al., 2001; Heath et al., 2001; Slakianos et al., 2003). Kinesins can transport newly synthesized virions or secretory vesicles containing either viral membrane proteins or even complete virions to the plasma membrane [Fig. 3(6)]. Kinesin-1 mediates the transport of vaccinia virus and African swine fever virus from the site of virus assembly to the plasma membrane (Rietdorf et al., 2001; Jouvenet et al., 2004). The vaccinia virus protein A36R interacts directly with the light chain of kinesin-1 (Rietdorf et al., 2001; Ward and Moss, 2004). However, vaccinia virus lacking A36R is also conveyed along microtubules to the cell periphery, albeit with reduced efficiency (Herrero-Martinez et al., 2005). Thus, vaccinia virus must encode other receptors for plus-end directed microtubule motors, the most likely candidate being the F12L protein (van Eijl et al., 2002; Smith et al., 2003). Other viral interaction partners for kinesins include the gag-polyprotein of many retroviruses and several herpes simplex virus proteins (Tang et al., 1999; Diefenbach et al., 2002; Koshizuka et al., 2005).

**Virus egress – getting out of town and surfing away**

After viral assembly has been completed, viruses leave the cell either one-by-one by exocytosis or budding from the plasma membrane [Fig. 3(7 and 8)], or after cell lysis by disruption of the plasma membrane which frees all progeny virus. The latter approach is used by adenoviruses and other non-enveloped viruses, and probably occurs for all viruses after cell death (Garoff et al., 1998). Leaving one-by-one again requires overcoming the actin cortex beneath the plasma membrane. As during virus entry, membranes containing entire virions may modify the actin cortex by similar mechanisms as the host’s secretory or exocytic vesicles. For example, melanosomes bind kinesin-1 and myosin V simultaneously, and can therefore switch tracks from microtubules to actin filaments (Seabra and Coudrier, 2004). This dual character of the actin cytoskeleton, either being a barrier or providing tracks, may explain why short-term treatment with actin depolymerizing drugs increases the release of equine infectious anaemia virus (Chen et al., 2004), while actin depolymerization and inhibition of myosin light chain kinase, which regulates myosin II activity, inhibit budding of HIV (Sasaki et al., 1995). Budding of filamentous influenza virus is also affected by actin depolymerizing drugs, probably because the actin cortex maintains the correct structure of lipid rafts that are incorporated into the viral envelope (Simpson-Holley et al., 2002).
Besides mimicking cellular cues, viruses can also reorganize the cytoskeleton and restructure the host transport machinery to suit their needs. Several viruses interfere with the microtubule nucleating activity of the MTOC, either by disrupting centrosomes as African swine fever virus and vaccinia virus do (Ploubidou et al., 2000; Jouvenet and Wileman, 2005), or by inducing the formation of supernumerary centrosomes as in hepatitis B virus infection ( Forgues et al., 2003; Yun et al., 2004). Human T cell leukemia virus and HIV alter microtubule organization to build a virological synapse promoting efficient cell-to-cell spread and rapid dissemination of these viruses within secondary lymphoid tissues in vivo (Igakura et al., 2003; Piguet and Sattentau, 2004; Nejmeddine et al., 2005).

Vaccinia virus exploits the actin cytoskeleton in a peculiar manner for efficient cell-to-cell spread. Intracellular enveloped virus (IEV) of vaccinia virus fuses with the plasma membrane, but remains attached as cell-associ-ated enveloped virus (CEV; Smith et al., 2003). Subsequently, the vaccinia protein BSR that is now inserted in the plasma membrane beneath the CEV activates Src family kinases (Newsome et al., 2004), which phosphorylate cytosolic tyrosine residues of the vaccinia virus membrane protein A36R that has also been inserted into the plasma membrane underneath the CEV upon IEV fusion (Frischknecht et al., 1999). This signal releases kinesin-1 from A36R and leads to the recruitment of an actin polymerization machinery, including the actin-nucleating complex Arp2/3 (Moreau et al., 2000; Scaplehorn et al., 2002; Newsome et al., 2004). The Arp2/3 complex induces actin polymerization [Fig. 3(9)], and allows the CEV to surf away from the infected cell (Smith et al., 2003). Actin tail formation is independently initiated by Src-family kinase and Abi-family kinases. The latter also induces the release of CEV from the cell to start another round of virus replication, and yet another journey through a cell (Reeves et al., 2005).

**Outlook**

During the development of viral vectors for efficient therapeutic gene expression, it became apparent that the transport of naked DNA to the nucleus, the site of transcription, is a major barrier for transfection, and hence for the application of human non-viral gene therapy vectors in vivo (Suh et al., 2003; Dauty and Verkman, 2005). However, several viruses have found elegant solutions for these tasks, and successfully negotiate with the cytoskeleton for assistance in the required transport steps. Moreover, the relationship between the different forms of intracellular viral travel, and the various interactions with the molecular motors of the myosin, dynein and kinesin families advance our understanding of the viral pathogen-esis. This may lead to the identification of new targets for the development of antiviral therapy with drugs that do not inhibit viral enzymes but specific host or virus-host inter-actions, and are thus less likely to promote the evolution of drug-resistant viral strains. Virus-based expression vectors should include all factors that modulate the host cytoskeleton during entry and transcription, but not those involved in virus replication and egress.

While many viruses infect polarized epithelial cells (Blau and Compans, 1996; Bergelson, 2003; Bomsel and Alfsen, 2003), the interaction between molecular motors and viral particles has mainly been studied using non-polarized cells. In polarized columnar epithelial cells, microtubules are longitudinally arranged with their minus-ends oriented towards the apical and their plus-ends pointing towards the basal plasma membrane (Müsch, 2004; Döhner et al., 2005). Therefore, in contrast to viral entry for example at the presynapse of neurons or at the plasma membrane of non-polarized cells, nuclear targeting after entry at the apical membrane of polarized epithelia would require a plus-end directed motor (Marozin et al., 2004). During assembly and egress, a minus-end directed microtubule motor might transport subviral components to the apical membrane. Moreover, after entry at the basolateral surface, minus-end directed microtubule motors would convey viral cargo to the apical compartments, if viral particles were not able to detach from the microtubules in due time to switch over to the nucleus. Future studies will dissect the function of molecular motors during infection of polarized epithelia.

Moreover, viruses have been trained as cell biologists for millions of years, and thus teach us unexpected molecular lessons about intracellular itineraries.

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