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Exploitation of cytoskeletal networks during early viral infection

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Abstract

Being dependent upon host transport systems to navigate the cytoplasm, viruses have evolved various strategies to manipulate cytoskeletal functions. Generally, viruses use the actin cytoskeleton to control entry and short-range transport at the cell periphery and exploit microtubules (MTs) for longer-range cytosolic transport, in some cases to reach the nucleus. While earlier studies established the fundamental importance of these networks to successful infection, the mechanistic details and true extent to which viruses usurp highly specialized host cytoskeletal regulators and motor adaptors is only beginning to emerge. This review outlines our current understanding of how cytoskeletal regulation contributes specifically to the early stages of viral infection, with a primary focus on retroviruses and herpesviruses as examples of recent advances in this area.

Keywords

Viruses; Actin; Microtubules; motors; trafficking; uncoating

Viral and Host Interplay in the Cytosol

Given the limitations to free diffusion in the dense cytosolic environment, viruses have evolved means to hijack their host's cytoskeletal macromolecular transport networks and the factors that regulate them in order to facilitate their movement. In past decades, an enormous body of work has provided evidence of and insights into the complex interplay of viruses and the host cytoskeleton both as virus particles enter and exit the cell, as well as during the variety of intracellular events that determine the outcome of infection. These include the reorganization of host organelles to form viral replication sites, generation of host antiviral responses and viral countermeasures, and the assembly, transport and release of new viral particles. These processes have been more broadly reviewed in recent years [1, 2]. In this review, we focus on recent advances in our understanding of how different viruses subvert two major cytoskeletal networks, actin filaments (or microfilaments) and microtubules (MTs), during the early stages of their replication. While we briefly discuss core strategies

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common to a variety of viruses for subverting actin filaments and MTs, we focus largely on retroviruses and herpesviruses as well-studied examples of RNA and DNA viruses, respectively, that utilize broadly divergent early infection strategies.

The host cell cytoskeleton

The host cytoskeleton consists of a scaffold of dynamic filaments that radiate throughout the cell. These adaptable arrays and their associated motor proteins facilitate a wide range of processes including reshaping plasma membranes, the capture and transport of cargos resident within or taken up by the cell, as well as the spatial organization of organelles that are central to cell shape, polarity, motility or division. These filaments are divided into three types: microfilaments, MTs and intermediate filaments [3, 4]. While intermediate filaments are primarily responsible for conferring mechanical stability to cells, the actin and MT cytoskeletons provide an active transport system for a variety of cargos, including infectious microorganisms such as viruses (Figure 1). Both actin and MTs form polarized filaments whose more actively growing plus-ends are, in general, pointed towards the plasma membrane and into cellular protrusions. This polarity provides a means for selective directional transport of cargos using ATP-driven motors toward one or the other end of the filament. Actin-mediated transport is driven by the myosin family of motor proteins, typically with myosin I or V transporting cargo towards the plus-end (also referred to as the barbed end) and myosin VI, and possibly IXb, transporting cargo towards the minus-end (also called the pointed end). Transport mediated by the actin cytoskeleton can alternatively occur via actin nucleation and/or polymerization, which is regulated by signaling through the Rho family of GTPases (including Rac, Cdc42, and RhoA) [3].

While microfilaments are generally responsible for short-range cargo transport mostly at the cell periphery, MTs are used for long-range intracellular transport. MTs are composed of α -/ β -tubulin heterodimers that assemble into cylindrical filaments. Their minus-ends are anchored at MT-organizing centers (MTOCs), the most dominant of which in many cell types is the centrosome (Figure 1). However, several lesser-studied yet important MTOCs also exist and help generate a complex and functionally diverse MT array within the cell [5]. MT-mediated transport is driven by two families of motor proteins, dynein and kinesin. Dynein moves cargo toward the minus-end (retrograde transport) while nearly all kinesins transport cargo toward the plus-end (anterograde movement). MT plus-ends radiate outward from MTOCs, forming filaments that dynamically grow or shrink. This dynamic behavior enables MT filaments to continuously explore the intracellular environment and engage targets such as organelles or cargos through a process known as “search and capture”. In many cell-types the majority of MTs are highly dynamic and have a short half-life of several minutes, but a small subset of these filaments can become stabilized and persist for several hours. In other cell types, such as neurons, vast networks of stable MTs exist. These long-lived filaments acquire distinguishing post-translational modifications including acetylation and detyrosination of α -tubulin, and can act as specialized tracks for cargo trafficking [4]. MT dynamics and stability are regulated by a variety of microtubule-associated proteins (MAPs), a subset of which are the highly specialized plus-end tracking proteins (+TIPs) [6]. Amongst these, the end-binding (EB) proteins act as master regulators of MT plus-end behavior; EB proteins specifically recognize GTP-tubulin that is transiently present at MT

plus-ends before hydrolysis to GDP-tubulin, and promote both MT polymerization directly and the recruitment of other +TIPs to MT plus-ends (Figure 1). Different +TIP complexes respond to different signaling cues which enables the cell to locally control MT stability at specific regions of the cell, driving cell polarization for example [6]. MT regulatory signaling pathways include Rac1, glycogen synthase kinase 3 β (GSK3 β) and RhoA-diaphanous (Rho-Dia), some of which also converge on actin and therefore play an important role in coordinating overall cytoskeletal dynamics in a variety of contexts.

Viral interactions with the actin network

One of the first cytoskeletal components encountered by viruses as they infect a cell is the actin network (Figure 2A). In one respect, the dense layer of microfilaments underneath the plasma membrane (termed the cortical actin meshwork) represents a physical barrier to infection that must be penetrated. On the other hand, viruses also exploit this actin network to facilitate various entry and post-entry processes [7]. But before they even do this, actin also influences the organization of surface receptors and membrane structures that mediate virus attachment, highlighting the complex role of actin in early infection.

While viruses attach through various surface receptors, some have to then search the cell surface to find entry spots. This movement is mediated by transmembrane receptor connectivity to the dynamic actin network just beneath the plasma membrane. For example, non-enveloped viruses including the RNA virus picornavirus or Cocksackievirus or the DNA adenoviruses (Ads), as well as enveloped viruses like hepatitis C virus (HCV), use this “cell surfing” to find entry receptors buried within tight junctions; this movement, receptor engagement and entry all involve actin-dependent processes [8–11]. Many RNA viruses, such as avian leucosis virus (ALV), murine leukemia virus (MuLV) and vesicular stomatitis virus (VSV), as well as both small and large DNA viruses ranging from human papillomavirus type 16 to a number of herpesvirus family members, “surf” along actin-rich protrusions called filopodia in a myosin driven fashion to reach entry hotspots [12, 13]. Herpesviruses serve as an example of how this surfing contributes to entry and the signaling pathways involved vary depending both on the virus and cell type in question. Herpes simplex virus-1 (HSV-1) infects neuronal cells by inducing dendritic filopodia formation, which virus particles can bind to facilitate their movement toward the cell body [14]. In addition to neuronal cells, HSV-1 induces filopodia in a wide range of cell lines in an actin-dependent manner that is regulated by viral receptors and virus induced Cdc42 signaling; however, this appears to involve RhoA in some, but Rac1 in other cell types [15–20]. Moreover, as discussed below HSV-1 entry is complex and involves additional receptor-mediated signaling pathways such as PI3K and p21-activated kinase that transiently control actin regulators such as cofilin [21–24].

Other viruses use actin remodeling to organize surface receptors. HIV-1 attachment concentrates its receptors (CD4 and CXCR4) at the cell surface to facilitate fusion with the plasma membrane in a manner that involves Rho-GTPase signaling and requires the actin-remodeling proteins, filamin-A and cofilin [25–29]. In addition, fusion of the HIV-1 envelope with the plasma membrane activates Rho-GTPase signaling pathways resulting in actin polymerization and capsid entry. This process also depends on additional actin

regulatory proteins such as Abl, Wave2 and Arp3, as well as the ERM (ezrin-radixin-moesin) family member, moesin that cross-links actin filaments with the plasma membrane [27, 30, 31]. The HIV-1 accessory protein Nef enhances infectivity at least in part by promoting membrane-fusion mediated viral entry through remodeling the cortical actin barrier to allow virus penetration [32, 33]. Another actin-remodeling protein, villin has recently been shown to be recruited by Sendai virus during its fusion with hepatocytes [34]. Other examples of viruses entering by direct fusion of virion membranes with the cell membrane are human parainfluenza virus 3 (HPIV-3) and human respiratory syncytial virus (HRSV). Although still not well understood, a dynamic actin network is involved in both entry and subsequent syncytium formation for both of these viruses [35–38].

The role of actin in receptor engagement and virus entry varies considerably because different viruses use different strategies to penetrate into the cytosol that include direct receptor-mediated fusion at the plasma membrane or low pH endosomal entry [39]. Viruses ranging from the large DNA virus, African swine fever virus (ASFV) to RNA viruses such as dengue virus, VSV or Ebola virus exploit actin-mediated endocytic pathways to infect a wide range of cell types [40–47]. Rabies virus, for example enters into epithelial cells through elongated clathrin pits and depends on actin to complete the entry process, as disrupting actin polymerization or the actin regulator, dynamin blocks virus entry [48]. A similar process allows rabies virus internalization into primary peripheral neurons and pH-dependent fusion of virions at the cell body [49]. Complicating matters further, some viruses including retroviruses and herpesviruses can employ multiple strategies to enter the cell. The β -herpesvirus, human cytomegalovirus (HCMV) exploits integrin receptor-mediated signaling through SRC tyrosine kinase and paxillin to control actin dynamics and endocytic entry into monocytes, but not fibroblasts [50]. The α -herpesvirus, HSV-1 enters many cell types by membrane fusion but is also capable of entry by endocytosis, phagocytosis or macropinocytosis, which is a form of actin-dependent membrane blebbing and endocytic engulfment often involved in fluid uptake [51]. In some cell types endocytosis is not involved but specific components of the macropinocytosis machinery are needed, yet because of the shared functionality of actin remodeling factors it remains difficult to discern whether entry is via fusion and/or macropinocytosis [24]. Similarly, receptor mediated activation of diverse signal pathways controlling actin dynamics mediate the endosomal or macropinocytosis-like entry of the γ -herpesvirus, Kaposi's sarcoma-associated herpesvirus (KSHV). Again, the routes of entry used depend on cell type, with macropinocytosis being controlled by PI3K signaling and regulatory mono-ubiquitination of actin and myosin IIA by host E3 ubiquitin ligase, c-Cbl during entry into endothelial cells [44, 52–55]. Other viruses, including the poxvirus vaccinia virus (VacV) also promote their entry by stimulating macropinocytosis [39, 56, 57].

Once inside the cell, many viruses will exploit actin polymerization as well as actin motors to mediate their early intracellular movements. Most RNA viruses replicate in the cytoplasm and their “early” stage completes quite rapidly without needing to reach the nucleus. However, post-entry movement of viruses such as poliovirus, a picornavirus, to establish cytoplasmic replication centers remains dependent on an intact actin network [58, 59]. The actin regulator cofilin also facilitates the formation of perinuclear measles virus ribonucleoprotein complexes that initiate viral RNA synthesis [60].

Some viruses exploit actin to prevent the phenomenon of superinfection, which blocks the entry of additional viruses into already-infected cells. Actin reorganization has been implicated in superinfection exclusion by viruses ranging from insect baculoviruses to cytoplasmically-replicating mammalian poxviruses [61–65]. After establishing infection, VacV expresses several proteins that recruit and activate host actin nucleators to form actin protrusions that exclude incoming viruses [61, 62, 64–66]. Interestingly, SRC-mediated actin nucleation forms projections from the cell surface that also enable egressing progeny VacV particles to switch from initial outward movement on MTs to actin-based propulsion into neighboring cells in order to spread infection [67–69], the opposite form of cytoskeletal hand-off to that which occurs during early post-entry infection. As such, remodeling of the peripheral actin cytoskeleton plays incredibly diverse roles in regulating virus entry both during primary infection and subsequent spread to infect neighboring cells.

Transition of viruses from actin network onto MTs

With a few exceptions of viruses that predominantly exploit actin-driven motility, most viruses switch from the actin cytoskeleton to MT tracks to promote long-range movement of their cores [1, 2]. Viruses entering the cell utilize MT networks in a variety of ways (Figure 2B). Some enter, at least initially, through endocytic vesicles that are transported by MTs and their motors. Even here, surface proteins initially involved in virus attachment continue to influence these early processes. After endosomal uptake the poliovirus receptor (PVR), for example, contains a cytoplasmic domain that interacts with dynein subunits and adaptors to control retrograde movement of vesicles [70, 71]. MTs also influence the escape of several viruses from endosomes to enter the cytosol. Other viruses that fuse into the cytosol at the plasma membrane must transit from actin networks to MTs, a hand-off process that often involves several host and viral proteins. This is evident by the fact that while little is known about the host proteins associated with incoming retroviral cores, both HIV-1 matrix (MA) and capsid (CA) proteins have been shown to associate with the actin and MT cytoskeleton, respectively [72, 73]. There are also multiple examples of host factors that regulate actin-MT cross-linking and negatively or positively influence infection by viruses. For example, early stages of infection by KSHV or HIV-1 require focal adhesion proteins and ERM family members [74–78]. Notably, both viruses induce MT stabilization during the early stages of infection and ERMs, for example, mediate actin-MT cross-linking that can capture and stabilize dynamic MT filaments [76, 77]. These stable MTs appear to be important for the translocation of HIV-1 and KSHV to the nucleus but, intriguingly, are not induced or utilized by other viruses for this purpose. However, HCV, which does not induce MT stability during early infection, is also negatively regulated by ERMs [79, 80]. This suggests that actin-MT cross-linking may also facilitate the transfer of virus particles to MTs, regardless of whether MT stabilization is involved. Beyond ERMs, the actin-MT interacting factor IQGAP1 binds to the MA domain of the retrovirus MuLV and promotes early infection [81].

For most RNA viruses the early stages of infection end and replication begins soon after entry into the cytoplasm. However, even for these viruses MTs play an important role in endocytic trafficking, the formation of replication compartments and the egress of new virions once infection is established [2]. For viruses whose ultimate destination is the

nucleus, most transport their genomes within intact capsid shells. HSV-1 capsids, for example, dock at nuclear pores and then inject their DNA into the nucleus. However, retroviruses such as HIV-1 are unusual; these RNA viruses reverse transcribe their genome into DNA that is ultimately integrated into the host chromosome. Reverse transcription promotes conical capsid (referred to as the capsid or core throughout this review) disassembly or “uncoating”. While hotly debated, growing evidence suggests that at least partial uncoating occurs in the cytoplasm during virus translocation to the nucleus, while additional likely also occurs at the nuclear membrane [82–85]. Indeed, actin and actin-MT cross-linking proteins have been implicated in regulating the HIV-1 uncoating process. Dia-related formins (Dia1 and Dia2) coordinate cytoskeletal remodeling by controlling both actin nucleation and MT stabilization. These formins were recently found to not only facilitate HIV-1-induced MT stabilization during entry but also to bind HIV-1 cores and regulate uncoating independently of their MT regulatory functions [86]. As such, Dia proteins appear to coordinate HIV-1 uncoating with the induction of MT stabilization and virus transport. As discussed below, opposing forces from MT motors also help in the HIV-1 uncoating process. Notably, another RNA virus that needs to reach the nucleus, influenza A virus also exploits antagonistic forces exerted by myosin II-driven transport on actin filaments and MT motor dynein complexes to regulate its uncoating [87]. The Influenza uncoating process is very different to that of HIV-1, with influenza proteins mimicking unfolded proteins to exploit the host’s aggresome machinery [87]. Collectively, these findings highlight the role of actin-MT crosslinkers in the transition of viral cores from peripheral actin to the MT network, and their specific roles in the unusual uncoating processes of both HIV-1 and Influenza.

Viral interactions with the MT network

Viruses often modulate MT dynamics to facilitate their “capture” and/or transport to the nucleus. One example is Ad, which activates Rac1, protein kinase A (PKA) and p38 mitogen-activated protein kinase (p38MAPK) signaling pathways to both increase MT growth toward the cell periphery and enhance dynein-mediated transport [88]. Stimulating the growth of MTs toward the cell periphery may facilitate the capture of incoming Ad particles [89] and/or promote the formation of the stable MTs [90], which involves filament growth and capture at sites such as the plasma membrane. There is growing evidence that in at least some cases, viruses do not randomly engage MTs but are, instead, captured by MT plus-ends as if they are cellular cargos. Studies of HSV-1 have shown that incoming virus particles require a plus-end localized complex of EB1 and the +TIP, cytoplasmic linker protein 170 (CLIP170) to initiate dynein-mediated transport to the nucleus after entry into the cytoplasm [91]. Although HSV-1 capsids bind dynein directly, they also bind the dynein adaptor Dynactin 1 (DCTN1) [92–94]. While this may enhance dynein-mediated transport, DCTN1 associates with EB1 and EB1/CLIP170 complexes and appears to play a critical role in the capture of incoming particles prior to initiating their dynein-mediated transport [91]. Foot-and-mouth disease virus (FMDV) colocalizes with DCTN3 during early infection and this is required for virulence in cultured primary cells and in cattle [95]. Intriguingly, unlike HSV-1 that enters by membrane fusion and exploits EB1 to initiate transport, Flaviviruses enter through an endocytic pathway that mediates the uptake of large cargos and

is dependent on lymphocyte antigen 6 locus (LY6E) [96]. During cargo or Flavivirus uptake, LY6E forms tubule-like structures in a manner dependent on EB3, but not EB1. The family members, EB1 and EB3 form hetero- or homo-dimers and are often functionally interchangeable. However, these recent findings in viral systems, which also include clear differences in their contributions to late stages of HCMV replication [97], add to growing evidence of functional diversification between EB family members. Moreover, they suggest that different viruses exploit different +TIP complexes as a means to engage MTs through their dynamic plus-ends, and future studies will undoubtedly determine whether this is a more universal or virus-specific strategy.

While HSV-1 uses +TIPs to load onto MTs, other viruses exploit these specialized host factors to influence MT dynamics. Here again, herpesviruses and retroviruses provide intriguing examples of diversity in strategies used even amongst virus family members; HSV-1 does not induce MT stabilization early in infection, but KSHV does. KSHV accomplishes this through the activation of Rho-Dia signaling, activated by integrin receptor engagement by the viral glycoprotein, gB [74, 75]. Amongst retroviruses, HIV-1 rapidly induces extensive MT stabilization during the early stages of infection, yet MuLV and simian immunodeficiency virus (SIV) do not [86, 98, 99]. Recent findings have begun to reveal the extent to which HIV-1 targets distinct +TIPs to accomplish this stabilization; the MA protein of incoming HIV-1 particles interacts with the EB1-associated +TIP Kif4 to induce MT stabilization soon after entry [99]. However, the levels of stable MTs in infected cells continue to increase over time. This appears to be because, following the loss of MA soon after entry, the HIV-1 capsid also induces MT stabilization. This occurs through the binding of the capsid to another family of EB1-associated +TIPs, the formins Dia1 or Dia 2 [86]. Intriguingly, although Dia1/2 regulate actin-MT cross-linking in uninfected cells, HIV-1 does not appear to utilize the actin-regulatory functions of these formins during early infection. Instead, the capsid binds Dia1/2 to facilitate uncoating as well as MT-based virus transport [86]. As uncoating occurs, it likely exposes more and more CA protein to the cytoplasm and drives further MT stabilization. Notably, Dia1/2 do not contribute to infection by MuLV or SIV, two retroviruses that do not induce MT stabilization. As such, through formin proteins HIV-1 elegantly coordinates its uncoating with the amplification of the stable MT subsets that it uses to reach the nucleus. Other host factors that promote early HIV-1 infection such as MT-associated proteins MAP1A and MAP1S have also been reported to interact with CA protein and facilitate MT stabilization [100], highlighting the extent to which HIV-1 proteins target MT regulatory proteins to ensure the formation of stable MT networks during early infection.

Many viruses directly engage motor proteins to enable their MT-based movement. HSV-1 capsids, for example, directly bind both dynein and kinesin, as well as the dynein processivity factor DCTN1 to regulate their bi-directional movement toward the nucleus [92, 93]. The hexon subunit of adenovirus directly binds dynein after exiting endosomes [101]. We direct readers to a recent review for more information on the wide array of viral interactions with MT motors [1]. However, some viruses including retroviruses appear to employ more indirect means of exploiting motors. Although earlier studies demonstrated MT- and dynein-dependent trafficking of HIV-1 towards the nucleus there is no evidence for a direct association between HIV-1 cores and motor proteins, unlike those reported for

human foamy retrovirus and bovine immunodeficiency virus [73, 102–104]. Despite this, recent work has established the importance of both dynein and kinesin motors in both the bi-directional motility and disassembly of the incoming HIV-1 core [105, 106]. Motor engagement and regulation appears to be mediated, at least in part, by the adaptors bicaudal (BICD2) for dynein and fasciculation and elongation factor 1 (FEZ1) for Kinesin-1 [106, 107]. Given that kinesins exhibit higher affinity toward post-translationally modified stable MT filaments, they may facilitate retrograde HIV-1 transport on stable MTs. Moreover, HIV-1 uncoating is believed to be intricately coupled to its bi-directional movement on stable MTs as inhibiting dynein, kinesin-1 or either of the adaptors, BICD2 or FEZ1, affects HIV-1 uncoating [105–107]. This suggests that uncoating may be assisted by “tug-of-war” forces generated by opposing motors on the same particle. The mechanism by which HIV-1 regulates kinesin-1 activity has also begun to emerge; FEZ1 phosphorylation at Serine 58 regulates its binding to kinesin-1, and this site is required for the nuclear translocation and uncoating of HIV-1 particles [106, 108]. Moreover, while HIV-1 does not induce global phosphorylation of FEZ1, the capsid binds to MT affinity-regulating kinase 2 (MARK2) and locally phosphorylates FEZ1 on the viral core [108]. As such, HIV-1 carefully controls kinesin-1 activity through recruiting both the adaptor FEZ1 and its regulatory kinase MARK2 to viral cores. Interestingly, the dynein adaptor subunit DCNT2 interacts with the MuLV pre-integration complex (PIC) that is exposed after uncoating and regulates infection in a manner dependent on the route of viral entry [109]. While functions for this interaction in virus motility are unknown, adaptors and MT regulatory proteins are also exploited by viruses in MT-independent processes [2].

As they journey to the nucleus, many viruses including adenovirus, herpesviruses and retroviruses accumulate at the perinuclear centrosome before the final transition to the nuclear membrane [2]. While this brief anterograde transport away from the centrosome is poorly explored for many viruses, HSV-1 appears to exploit +TIPs such as dyx1c1 to make this transition to the nuclear membrane [110]. Other viruses such as adenovirus and HIV-1, which exhibit fast MT-based transport towards the nucleus, seem to switch to a slower MT-independent motion prior to nuclear entry [103, 111]. Kinesins have also been implicated in the docking and uncoating of Ad and HIV-1 particles at the nucleus [112, 113]. Finally, the only mammalian DNA viruses that do not need to reach the nucleus for replication, poxviruses and asfviruses, need MTs after entry to organize their viral replication compartments in the cytoplasm [2].

Concluding Remarks

As parasitic cargos, viruses are adept hijackers of their host’s cytoskeletal networks. Recent studies have provided a wealth of insight into how viruses coopt both regulatory signaling pathways and specialized cytoskeletal regulators to coordinate the complex interplay between actin and microtubule networks in order to facilitate infection. While this review focuses on early stages of infection, particularly by retroviruses and herpesviruses to illustrate key points, a wide range of viruses exploit these networks to facilitate their diverse replication cycles that occur at different subcellular sites and widely differing cell types. Moreover, the insights gained from viral systems continue to add to our broader understanding of how cytoskeletal networks are regulated and function.

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Outstanding Questions

- How exactly do viruses regulate their transition between actin and MT filaments, and what are the host factors involved? Actin-MT cross-linkers appear to be involved, but it remains unclear whether they provide physical connections for virus particles to both networks, or promote actin-MT connections locally to facilitate virus capture or localized stabilization of MT filaments where virus particles are entering the cell. Recent studies of retroviruses and herpesviruses point to important roles for MT +TIPs in mediating the capture of virus particles and virus-induced MT stabilization. However, it remains to be determined whether this is unique to these viruses or a more universal phenomenon.
- Why is there bi-directional movement for so many viruses? Does this reflect a choice on the part of the virus and, if so, why? Does this allow viruses to randomly explore the cytoplasm or “back up” upon encountering roadblocks? For some viruses such as retroviruses evidence suggests bi-directional movement as a means to induce uncoating. But in some cases, does this represent an antiviral response attempting to expel the invading pathogen?
- Why do some viruses utilize, as far as we can tell, adaptors rather than directly binding to motor complexes? Perhaps this offers a means for more refined control of motor activity.
- Why do some viruses from the same family opt to stabilize MTs during entry, while others do not? Again, is this an active choice on the part of the virus or actually a host response?

Highlights

- Actin functions in a wide range of attachment and entry processes that are critical to the very earliest stages of virus infection. Actin remodeling also prevents superinfection, whereby viruses are prevented from entering cells that are already infected.
- Once inside the cell, virus particles transition from actin to microtubules (MTs) through actin-MT cross-linking factors and specialized MT plus-end tracking proteins (+TIPs) to initiate retrograde transport to the nucleus.
- Many viruses modulate MT dynamics by targeting regulatory signaling pathways and/or +TIP complexes to promote early infection.
- Interactions between viral capsids and MT motors and/or adaptor proteins regulate the bi-directional motility of incoming viral particles
- Opposing forces exerted by cytoskeletal motors plays a role in capsid disassembly or “uncoating” of some viruses.

Figure 1. Actin and MT organization in the cell.

Actin filaments (blue) form networks that regulate cargo transport at the cell periphery, as well as cell motility and shape. This includes the formation of filopodia (Inset Box 1) that are exploited by some viruses. MTs nucleate at the centrosome (orange) as well as sites such as the Golgi (brown). Growing MT plus-ends (green) radiate toward the cell periphery (Inset Box 2). Subsets of MTs can be captured at the cell periphery and become stabilized (red). Stabilized MTs can be used to transport cargos (yellow) to specific regions of the cell to generate polarity. **Inset Box 1:** Cortical actin interacts with MTs through a variety of

mechanisms including actin-MT cross-linking proteins (gray) or +TIP complexes, which can result in MT stabilization. Specific kinesin motors (white) recognize tubulin post-translational modifications to transport cargos on stable MTs. Myosin motors (dark blue) transport cargos on actin filaments. **Inset Box 2:** GTP-tubulin (yellow) is transiently present at MT plus-ends before hydrolysis to GDP-tubulin. EB protein dimers (orange) recognize GTP-tubulin at growing MT plus-ends and recruit other +TIP family members (blue). Dynein (red) mediates retrograde (minus-end directed) while kinesins (white) mediate anterograde (plus-end directed) cargo transport on MTs.

Figure 2. Viral interactions with the host cytoskeleton during entry and early infection. (A) Actin-mediated surfing. Viruses attach to transmembrane surface receptors (yellow). Actin (blue) dynamics and myosin motors mediate cell surfing by several viruses until they reach entry hotspots or sites where entry receptors (purple) are located, such as at tight junctions between cells. MTs are in red. (B) HIV-1 and HSV-1 serve as examples of divergent early infection strategies used by viruses. **Left: 1.** HIV-1 consists of a conical capsid (green) that encase its RNA genome, surrounded by MA protein shell (purple) within a lipid envelope studded with viral glycoproteins. HIV-1 induces MT stabilization very early in infection, which is regulated in part by actin-MT cross-linking proteins (gray). **2.** Fusion of HIV-1 into the cytosol releases MA protein (purple) that targets EB1 (orange) and Kif4 (blue) to induce MT stabilization. The capsid or CA protein (green) also interacts with the formins, Dia1/2 (red) as well as MAP1A (yellow), respectively, to induce additional MT stabilization. **3.** As uncoating occurs, CA protein release likely further increases MT

stabilization over time. **Bottom: The** capsid binds FEZ1 (dark blue) and BICD2 (gray) to bridge viral particles to kinesin-1 (white) and dynein (red) motors, respectively, to mediate their transport on stable MTs to the nucleus. Notably, reverse transcription of the viral RNA genome into cDNA occurs in a manner tightly coupled to uncoating. After docking with the nuclear envelope, the viral genome is delivered into the nucleus and integrates into host chromosomes. **Right: 1.** HSV-1 consists of an icosahedral capsid encasing its dsDNA genome (red), surrounded by a lipid envelope studded with viral glycoproteins. **2.** After fusion into the cytosol, dynamic MTs capture HSV-1 capsids through a plus-end localized complex consisting of EB1 (orange), CLIP170 (blue) and DCTN1 (green). **3.** This plus-end capture initiates motor-mediated retrograde transport, and does not involve stabilization of MTs by the virus. Note, HSV-1 capsids do not uncoat like HIV-1. Once capsids dock at nuclear pores the viral DNA genome is injected into the nucleus and maintained as an episome. **Bottom:** HSV-1 capsids directly interact with DCTN1 (gray), dynein (red) and kinesin-1 (white), which mediate the directed movement of HSV-1 to the nucleus.