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Retroviral proteins that interact with the host cell cytoskeleton

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Summary

In the past decade, several lines of evidence have highlighted the importance of the host cell cytoskeleton in various stages of retroviral infection. To complete their lifecycle, retroviruses must penetrate the outer barrier of the cell membrane, and viral cores containing the viral genome must traverse the cytoplasm to the nucleus and then viral gene products must make the journey back to the cell surface in order to release new progeny. The presence of a dense cytoskeletal network and organelles in the cytoplasm create an environment that greatly impedes diffusion of macromolecules such as viruses. As such, retroviruses have evolved means to hijack actin as well as microtubule cytoskeletal networks that regulate macromolecular movement within the host cell. Developing studies are discovering several host and viral factors that play important roles in retroviral trafficking.

Introduction

Retroviruses

Retroviruses are intracellular parasites that cause a wide variety of diseases ranging from malignancies and neurological disorders, to acquired immunodeficiency syndrome (AIDS) caused by the human immunodeficiency virus type 1 (HIV-1). All retroviruses contain three major structural genes (*gag*, *pol*, *env*) and are further described as simple or complex depending upon the presence of additional genes. The genomes of simple retroviruses such as murine leukemia virus (MLV) consist of the three structural genes, whereas more complex retroviruses such as HIV-1 and simian immunodeficiency virus (SIV) contain additional regulatory and accessory genes. Retroviral virions contain two identical positive-sense, single-stranded RNA genomes contained within a viral particle of matrix (MA), capsid (CA), and nucleocapsid (NC) proteins which are formed by proteolysis of a Gag precursor protein during virion assembly. The *pol* gene products packaged into the viral core consist of reverse transcriptase (RT) responsible for conversion of viral RNA into DNA, protease (PR) responsible for processing viral precursor proteins to their mature products, and integrase (IN) that inserts the viral DNA into the host genome. The core is surrounded by a cell membrane-derived envelope and glycoproteins encoded by the *env* gene. Traditionally, the retroviral life cycle is divided into two distinct stages. The early phase refers to virus attachment, fusion (either at the plasma membrane or at the periphery of the cytoplasm) and release of the internal viral core into the cytoplasm, followed by uncoating or disassembly of the core, reverse transcription of viral

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RNA into double-stranded cDNA within the viral reverse transcription complex (RTC), maturation of the RTC to a preintegration complex (PIC), translocation of the PIC to the nucleus and incorporation of the viral genome into the host genome as a provirus. The late phase extends from the expression of viral genes, assembly, budding and maturation of progeny virions [1] (Figure 1).

Both early and late phases of the retroviral lifecycle are intimately linked to and dependent upon the host cell cytoskeleton. From the point of view of infectious retrovirus, the host cell cytoskeleton is both an impediment to be overcome, posing as a physical barrier during processes such as virus entry and egress, and a track to facilitate intracellular motility required for a successful infection.

Host cell cytoskeleton

Due to the dense and viscous nature of the cytoplasm, active mechanisms are required for directed transport of host organelles and macromolecules [2]. The cytoskeleton consists of a scaffold of filaments within the cell cytoplasm. These filaments are dynamic and are divided into three types: actin (or microfilaments), microtubule, and intermediate filaments. While the intermediate filaments primarily provide mechanical stability to cells, the actin and microtubule cytoskeletons are responsible for trafficking of numerous endogenous cargos, as well as intracellular microorganisms such as viruses throughout the cell (reviewed in [3,4*] and [5]). Both actin filaments and microtubules form polarized filaments to which monomeric subunits are added at a growing plus end (towards the plasma membrane) in a highly complex regulated process [6]. Since both microfilaments and microtubules have polarity they are able to selectively transport their cargo to their respective termini using ATP driven motors that move in the same direction. Actin directed transport is driven by the myosin family of motor proteins, with plus-end directed myosins (myosin I or V) transporting cargo towards the cell periphery and minus-end directed myosins (myosin VI and possibly IXb) transporting cargo in the opposite direction [7]. Alternatively, transport mediated by actin systems can occur via actin nucleation, by newly polymerized actin filaments pushing a particle [6]. Generally, actin filaments are responsible for short ranged transport of cargo most frequently at the cell periphery while microtubules are used for long ranged transport throughout the cell. In general, microtubule plus-ends point towards the plasma membrane, where they can interact with the actin cortex (highly concentrated actin underneath the plasma membrane) while the minus-ends are stabilized by binding to a perinuclear area termed the microtubule-organizing center (MOTC), or to other minus-end binding proteins [8]. Microtubule-mediated motion is driven by the dynein and kinesin families of motor proteins. Kinesins typically transport cargos towards the periphery of the cell whereas dynein motor complexes drive minus end directed motion [9]. What determines the cargo specificity is not fully understood. However, the dynein cofactor dynactin is required for dynein function and has been shown to facilitate the interaction between dynein and membrane-bound cargo [10].

Viral Interactions with the cytoskeletal network

The limitations to free diffusion in the cytoplasm have forced viruses to evolve efficient mechanisms to manipulate the transport systems of their hosts. Almost all viruses utilize the cytoskeleton for their transport within cell during infection. Despite this, little is known about the detailed mechanisms by which viruses exploit cytoskeletal dynamics. In almost four decades, techniques such as high resolution microscopy of infected cells and specific tools to modulate cytoskeletal architecture have led to more insight into the complex interplay of retroviruses and the host cell cytoskeleton. Recent developments with a focus on individual steps of the retroviral life cycle are discussed in this review.

Early Stages of Infection

(a) Fusion and entry: To enter the cell, viruses need to penetrate the barrier posed by the dense layer of actin filaments underneath the plasma membrane, termed cortical actin. The route of entry (endocytosis or fusion) varies depending upon both the viral envelope and the cell type being infected [11]. Some retroviruses, such as the MLVs and the avian leucosis virus (ALV), enter via endocytosis and deliver their viral capsid upon endosome acidification and fusion in the cytoplasm [12] (Figure 1). Although endocytosis can naturally bypass the cortical actin barrier, there is a chance of subsequent viral degradation in low pH compartments, which probably explains why other retroviruses, such as HIV-1, enter in a pH-independent manner by fusion at the plasma membrane and deposition of the viral core at the periphery of the cytoplasm [13]. One of the first viral proteins identified that had an impact in cytoplasmic trafficking of virions in target cells was the HIV-1 accessory protein Nef (reviewed in Anderson and Hope 2005) [14]. Nef increases viral infectivity early in replication but only when virions are pseudotyped with pH-independent envelope proteins, such as that of HIV-1, that fuse at the plasma membrane [15]. Recent findings suggest that Nef can remodel the cortical actin barrier to allow the virus to penetrate, as disruption of the actin cytoskeleton in target cells compensates for the absence of Nef during infection only when virus enters the cell by membrane fusion but not when entry is by endocytosis [16**].

Although the actin cytoskeleton functions as a barrier for the entry of incoming viruses [16**] some studies have shown actin to facilitate early retroviral trafficking [17**,18]. Even prior to entering the cell, in an actin-myosin driven movement, MLV and several other retroviruses pseudotyped with the vesicular stomatitis virus (VSV-G) envelope protein surf along filopodia to regions of the cell that are vulnerable to viral entry [17**]. In addition, clustering of primary HIV-1 receptor CD4 and CXCR4 coreceptors and activation of Rho signaling by actin microfilaments have been suggested to facilitate viral fusion [18,19]. Furthermore, increased levels of stabilized acetylated microtubules (MTs) [20] induced by viral entry have also been suggested to play a role in HIV-1 fusion [21].

(b) Short distance actin trafficking onto microtubules: Once inside the cell, retroviruses also use cortical actin for short distance transport of viral genomes from the peripheral regions of the cell to the microtubule network, where reverse transcription and longer ranged transport to the nuclear periphery occurs [11,22-25]. Live-microscopy indicating microtubule-independent trafficking of HIV-1 cores at the cell periphery after entry further supports this proposed initial short-range motion mediated by actin microfilaments [22,23]. It seems, therefore, that cortical actin facilitates post-entry movement of viral cores. Again, Nef-mediated actin reorganization appears to play an important role in the cytoplasmic trafficking of HIV-1 core at this early stage. The presence of Nef in the virions, its association with the viral core after entry and its interaction with proteins involved in actin cytoskeleton dynamics, such as Vav (a cellular Rho GTPase exchange factor) and PAK (a member of the p21-activated kinase family) are consistent with the notion that Nef may facilitate postfusion trafficking of the viral core via an actin-dependent mechanism (reviewed in Anderson and Hope 2005) [14**].

Little is known about the proteins associated with the retroviral genome in RTCs or PICs. For HIV-1, the viral matrix and CA proteins in RTCs have been shown to associate with the actin cytoskeleton [22] and microtubules [23], respectively, suggesting that they may function in actin-mediated movement and the transition of viral cores to the microtubule network. Recent work has shown that moesin, a member of the ezrin-radixin-moesin (ERM) family of cytoskeletal regulatory proteins, inhibits replication of both MLV and HIV-1 prior to reverse transcription by downregulating stable microtubules [26]. Stable microtubules have been suggested to function as specialized tracks for vesicle and cytoskeletal trafficking [27] and are

speculated to be involved in early step of HIV-1 [21] as well as non-retroviruses such as Kaposi's sarcoma-associated herpesvirus infection [28,29*]. The effects of moesin on retroviral replication at this early stage in the lifecycle suggest that viral cores rapidly transit from cortical actin to the microtubule network, allowing reverse transcription and movement towards the nuclear periphery. Understanding whether moesin plays a direct role in viral replication or inhibits replication through its effects on stable MT formation will provide important insights into the cortical actin-microtubule transition. Recent work indicates that IQGAP1, an actin and microtubule-interacting protein, binds to the MA protein of MLV and plays a positive role in both the early and late steps of viral replication [30]. Such cellular factors may facilitate the transition of cores to the MT network.

(c) Long distance microtubule trafficking to nucleus: The switch from actin microfilaments to microtubule tracks is thought to facilitate movement of cores to the cell centre. It has been indicated that both the human foamy retrovirus (HFV) and HIV-1 utilize the dynein-dynactin motors to move along microtubules and accumulate their RTCs/PICs in the perinuclear area or MTOC in infected cells [23,31,32]. Microtubule-dependent trafficking of retroviral Gag protein has also been described during later stages of the HTLV-I life cycle [33]. The interaction of HIV-1 IN, also present in RTCs/PICs, with yeast microtubule-associated proteins is consistent with microtubule-based trafficking to the nucleus [34]. In addition, overexpression of FEZ-1, a microtubule-associated neuronal transport protein, blocks the transport of retroviral DNA into the nucleus after reverse transcription [35]. Recent data suggests that FEZ-1 regulates the activity of the microtubule motor protein kinesin-1, affecting MT motility [36*]. Although kinesin-1 typically exhibits plus-end directed microtubule motion, it can also interact with proteins involved in nuclear import such as RanBP2 [37], suggesting that FEZ-1 interferes with MT-dependent processes at the nuclear periphery that allow entry of viral DNA into the nucleus.

Dynein transports the retroviral core only as far as the MTOC. The mechanisms for traversing the gap from the MTOC to the nuclear pore and any requirements for active transport across this region are poorly understood. For entry to the nucleus, simple retroviruses such as MLV require cell division and breakdown of the nuclear envelope whereas more complex viruses such as HIV can infect quiescent, nondividing cells (reviewed in [38]). The mechanism by which PICs are actively passed through nuclear membrane of target cells remains unclear. For HIV, MA, Vpr, IN and a DNA element formed during reverse transcription known as the central DNA flap have been reported to be involved in nuclear import activity of the PICs (reviewed in [39]). Recent efforts have cast considerable doubt on the involvement of any of these factors in nuclear import. A new proposal is that the HIV PIC is carried into the nucleus in association with tRNAs [40]. Whatever the route of entry, once provided access to the host genome, the PIC can mediate the formation of the integrated provirus.

Late Stages of Infection

(d) Assembly at the plasma membrane and budding: After viral RNA transcription and protein synthesis, the virion components need to be transported and accumulated at one common subcellular location to allow particle assembly and release. Studies on HIV nuclear export indicate a requirement for nuclear actin for exit of the HIV genomic RNA to the cytosol [41,42] where the viral Gag precursor protein is then synthesized and tethers viral RNA to actin filaments near the MTOC [43]. While fractions of Gag are reported to traffic in and out of the nucleus [44], Mason-Pfizer monkey virus (MPMV) Gag is also transported to the MTOC for particle assembly [45,46]. Recently, hnRNP A2 has been suggested to play a role in trafficking of the HIV-1 genomic RNA to the MTOC [47]. Gag may therefore bind viral RNA at MTOC rather than the nucleus, and then mediate its transport to the cell surface. Multimerisation of Gag exposes a hydrophobic myristic acid increasing its affinity for membranes [48]. Early

studies indicated that HIV-1 Gag could utilize actin microfilaments for transport to the plasma membrane of infected cells, potentially promoting assembly via interactions with actin [49, 50]. MLV Gag might reach the plasma membrane by direct interaction of its MA protein with the kinesin motor protein KIF4 [51], which has also been shown to associate with Gag of a number of other retroviruses [52]. Recent evidence also suggests that association of the actin and microtubule-binding factor IQGAP1 with MLV MA protein plays a role in virus assembly [30]. Initial electron microscopy studies indicate that budding of the mouse mammary tumor virus (MMTV) and Rous sarcoma virus (RSV) from cells occurs via surface protrusions containing actin filaments [53]. Also, actin is present in virion particles of both MMTV [53] and HIV-1 [54] and plays an important role in the budding of these viruses [55,56]. Besides actin, other cytoskeletal elements such as cofilin and actin-binding proteins ezrin and moesin are also present in newly generated infectious progeny HIV-1 (reviewed in [57]). Although not specifically addressed so far, actin might again serve both as a barrier [58] or providing tracks [56] for retroviral release. Finally, the role of cytoskeleton in retroviral lifecycle is also evident after budding as the formation of Env-induced virological synapses which increase the efficiency of both HIV-1 and HTLV-1 transmission from cell to cell is actin dependent [59] and involves reorganization of microtubules [60]. Also, modulation of actin dynamics has been proposed as an important mechanism for Nef-induced alterations of T cell receptor signaling in HIV-1 infection (reviewed in [57]).

Conclusion

It has become increasingly apparent that numerous retroviruses utilize the host cell cytoskeleton throughout their entire lifecycle. Generally, they use actin microfilaments for short ranged transport associated with events at the cell periphery such as viral entry and egress while they seem to hijack microtubule motors dyneins and kinesins for long ranged transport on the way in or out of the cell, respectively. Despite this, the detailed mechanisms by which retroviruses exploit cytoskeletal dynamics are still poorly understood. Earlier studies mostly indicated direct interactions of retroviral proteins with the host cytoskeleton, while more recent screens have identified several host cytoskeletal regulatory factors involved in intracellular trafficking of various retroviruses including HIV-1. This growing list of host/viral cytoskeletal regulatory proteins suggests that more such factors are yet to be identified. Understanding this parasitic relationship of retroviruses with the host cytoskeleton not only provides invaluable insights into host-virus interactions but may also uncover new strategies for the treatment of retroviral infection.

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Figure 1.

A schematic view of the interaction of retroviruses with the host cytoskeleton throughout their replication cycle. The incoming viral cores are deposited in the cytoplasm after entry by fusion at the plasma membrane followed by penetration of cortical actin (X) or by endocytosis which bypasses the actin barrier. The incoming cores are then transported towards the nucleus by short-range actin-mediated movement. Subsequently, the RTCs and the PICs move along microtubule tracks utilizing dynein motors (indicated as green arrows) towards the MTOC. Following nuclear import, integration and generation of viral proteins, particle components are transported again along MTs (most likely utilizing KIF4 motor) to the plasma membrane where they are assembled with the viral genome and penetrate through cortical actin as immature particles. Maturation of virions leads to generation of infectious progeny containing several cytoskeletal regulatory proteins. Cellular cytoskeletal regulatory factors and the stages at which they interfere with the retroviral life cycle are indicated at the right hand side (factors in black affect both MLV and HIV-1; green indicates HIV-1 specific factors and blue are MLV specific factors). Retroviral proteins that interact with the cytoskeleton are indicated.