Review

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HIV trafficking in host cells: motors wanted!

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Throughout the viral replication cycle, viral proteins, complexes, and particles need to be transported within host cells. These transport events are dependent on the host cell cytoskeleton and molecular motors. However, the mechanisms by which virus is trafficked along cytoskeleton filaments and how molecular motors are recruited and regulated to guarantee successful integration of the viral genome and production of new viruses has only recently begun to be understood. Recent studies on HIV have identified specific molecular motors involved in the trafficking of these viral particles. Here we review recent literature on the transport of HIV components in the cell, provide evidence for the identity and role of molecular motors in this process, and highlight how these trafficking events may be related to those occurring with other viruses.

Viral hijacking of cytoskeletal transport systems

The cytoplasm of eukaryotic cells is a complex and molecularly crowded environment [1] in which proteins move primarily by diffusion. In the case of larger macromolecules and organelles, or on the need to translocate from one compartment to another, proteins can attach to the cytoskeletal network and undergo active directed movement along filaments. Viruses fit both requirements for active cytoplasmic transport because their components are often too large to diffuse freely within the cytosol and they need to reach their site of replication rapidly. As such, viruses operate as molecular imposters that use well-established cellular processes [2–8]. In particular, viruses that replicate in the nucleus, such as adenoviruses [9,10] parvoviruses [11], retroviruses [12,13], and herpes viruses [14,15], use the microtubule network and associated motors to traffic within the cytoplasm either from the cell periphery to the center of the cell (dynein) or in the reverse direction (kinesins) (Box 1). Motility on actin filaments with the involvement of actin-driven motors (myosins) has also been described for retroviruses [13,16] and other viruses [17,18]. To date, much of the data surrounding the role of molecular motors during retroviral trafficking has been understood through studies of the lentivirus HIV.

Both the early and late phases of HIV viral replication are highly regulated events likely to require different molecular motors and numerous actin- and tubulin-associated proteins [19] (Table 1) at various stages (Figure 1) [20]. During the early phase, incoming virions (Box 2) fuse with the host cell membrane, allowing the viral complex referred to as reverse transcription complex (RTC) to be transported through the cytoplasm toward the nucleus. On successful conversion of the viral RNA into DNA, the complex, then referred to as a pre-integration complex (PIC) and devoid of capsid, enters the nucleus where it integrates its genome into host-cell DNA. During the late phase, the transcribed viral RNA is exported to the cytoplasm where it drives the synthesis of new viral proteins. These viral components are then transported in a coordinated fashion to the site of virus assembly leading to the release of newly formed virions.

With new technologies including gene editing, improved microscopy techniques, and high-throughput screening, the identification of molecular motors involved in the retroviral cycle and their characterization is achievable. This review discusses our current understanding of retroviral cytoplasmic trafficking during host cell infection, with an emphasis on HIV trafficking, and the questions remaining to be addressed. We also discuss HIV interaction with the cytoskeleton and associated motors in both the early and late phases of the replication cycle and compare this with other retroviruses or other virus families for which data are available.

HIV translocation: from plasma membrane to nuclear pore

The hallmark of all retroviral infections is integration of the viral genome into host-cell chromatin. For most retroviruses, such as murine leukemia virus (MLV) or human foamy virus (HFV), this requires nuclear envelope breakdown during prometaphase. By contrast, lentiviruses are actively imported to the nucleus via nuclear pore

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Box 1. Cytoskeleton and motors

The cytoskeleton is a dynamic assembly of structures that regulates cell shape and movement, chromosomal segregation, cell division, and intracellular trafficking of molecular complexes and organelles. It comprises three types of network: microtubules, intermediary filaments, and actin filaments, also called microfilaments. Microtubules are hollow tubes measuring ≈25 nm in diameter and several microns in length, comprising 13–15 protofilaments of α/β tubulin dimers arranged in parallel [93]. They undergo continuous assembly and disassembly, a process known as treadmilling, with the growing plus end oriented toward the cell periphery and the minus end toward the MTOC. Microfilaments are thinner filaments (≈7 nm diameter) comprising two parallel strands of actin monomers [94]. They are found throughout the cell but are particularly abundant beneath the plasma membrane, where they form an actin cortex. Similarly to microtubules, microfilaments are polar dynamic filaments that can ensure directed transport. By contrast, intermediate filaments are essentially involved in cell shape and movement and are generally not involved in long-range movement of cargos.

Myosins belong to a superfamily of mechanoenzymes sharing a conserved motor domain that is responsible for actin binding and for power force production through ATP hydrolysis and conformational change [95]. The myosin tail domain mediates interaction with cargos and, in certain cases, other myosin motors. Myosins are involved in the regulation of the actin cytoskeleton dynamics, in the control of cell shape and movement, and in the transport of many different cargos including organelles.

Kinesins belong to a superfamily that shares a motor domain able to bind microtubules in an ATP-dependent manner [95,96]. Typically, active kinesins also possess a coiled-coil domain that mediates homo/heterodimerization and enables their association with other regulatory subunits. Most kinesins move towards the plus end of microtubules, although a few kinesins move in the opposite direction. They possess divergent tail domains with various cargo-binding specificities. It allows them to transport particular cargos such as proteins, protein complexes, vesicles, or organelles.

Cytoplasmic dynein is a very complex molecular motor comprising two heavy chains, two intermediate chains, and several light and light intermediate chains [95,96]. This motor typically functions in conjunction with the dynactin complex, which comprises at least ten additional proteins and participates in motor processivity and cargo binding. Cytoplasmic dynein is responsible for the transport of many cellular cargos towards the minus end of microtubules.

Many cellular cargos exhibit bidirectional movement on microtubules due to their simultaneous attachment to dyneins and kinesins. Thus, opposite-polarity motors can be engaged in a tug-of-war (Figure 1, white arrows) [28]. These opposite-polarity motor activities are coordinated, resulting in proper spatial and temporal sorting and in the establishment and maintenance of the subcellular organization.

complexes (NPCs), which allows them to infect both dividing and non-dividing cells [21]. Because the nuclear entry site differs between lentiviruses such as HIV and most other retroviruses, it is likely that they also employ different trafficking pathways toward this site. Therefore, although our understanding of HIV trafficking can be strengthened by studies of other retroviruses, similar pathways and mechanisms cannot necessarily be inferred.

After cell entry, evidence suggests that microtubule-directed trafficking propels HIV RTCs toward the nucleus; single-particle tracking of incoming HIV revealed rapid and directed movement over several microns with burst velocities of the order of 1 μm/s [12,13]. In addition, treatment with nocodazole, which induces the disruption of the microtubule network, leads to the accumulation of viral complexes at the cell periphery and hinders infection [13,22]. With regard to MLV and HFV, incoming viruses traffic to and accumulate at the Microtubule-Organizing Center (MTOC) until nuclear envelope breakdown at the onset of mitosis [23–25]. Because chromosomes interact with the mitotic spindle during chromatid separation, accumulation at the MTOC may be an efficient mechanism allowing viral genomes to quickly reach chromosomes on nuclear envelope breakdown. As reported for HFV, retroviral Gag proteins may further assist both tethering of the viral DNA to the host cell chromatin and integration [26]. By contrast, lentiviruses access the nucleus via NPCs and, although RTC accumulation at the MTOC has been reported [12,13,27], the physiological relevance of this accumulation is unclear because it may prevent or delay nuclear entry by localizing viruses away from NPCs [13,27].

To properly engage and traffic along microtubules, some retroviruses probably rely on molecular motors of both the dynein and kinesin families. Single-particle tracking in living cells exposed to fluorescent viruses revealed bidirectional microtubular displacement, characterized by a series of saltatory retrograde and anterograde movements with overall directionality toward the nuclear compartment [13]. Retroviruses could therefore bind to molecular motors of opposite polarity sequentially or simultaneously as has been proposed for herpes simplex virus type 1 (HSV-1) [15], and undergo a tug-of-war phenomenon [28] with overall movement toward the center of the cell (Box 1 and Figure 2). The minus-ended motor dynein is thought to account for HIV and HFV movement toward the nucleus because treatment with a dominant-negative inhibitor of the p150 CC1 domain of dynactin (which uncouples dynein-based transport) or microinjection of antidynein antibodies led to clustering of viruses in the cell periphery [12,13,23]. Furthermore, a direct interaction between HFV Gag and LC8, the light chain of dynein, has been demonstrated [23]. However, recent evidence suggests that interaction with LC8 might be involved in other cellular processes and thus is not specific for retrograde transport [29]. Although microfilaments are also thought to be involved in trafficking incoming HIV [13,30], no functional interaction with myosin motors has been demonstrated. The difficulty in identifying direct HIV–motor interactions that specifically mediate retrograde movement could be due to the size and complexity of the motors, which can comprise several heavy and light chains and work in conjunction with other proteins. It may also be due to the functional redundancy of family members (particularly for kinesins) or to the fragile nature of the HIV capsid, which renders interaction approaches complex [31].

The precise composition of the HIV RTC that interacts with the cytoskeleton during the early phases of infection has been a source of debate. Other viruses that traffic to the nucleus interact with the cytoskeleton and/or with molecular motors via their structural shell. For example, the adenovirus capsid hexon interacts with cytoplasmic dynein intermediate and light intermediate chains [32], the herpes virus inner tegument protein VP1/2 recruits the dynein/dynactin motor complex [15,33], and HFV Gag interacts with dynein light chain 8 [23]. In the case of HIV, however, the field has been divided regarding the structure and composition of the trafficking complex. Some support the view that it no longer contains viral capsid