Unchain my heart, baby let me go—the entry and intracellular transport of HIV

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In this issue, McDonald et al. describe the itinerary of the incoming human immunodeficiency virus (HIV)* during its travels to the host nucleus. They show that subviral particles tagged with the green fluorescent protein (GFP) are propelled along microtubules (MTs) by minus-end–directed and presumably plus-end–directed MT motors. The tracked particles correspond to functional units, since they no longer include a viral envelope, but do include viral matrix protein, Vpr, capsid protein, and reverse transcription activity.

Opening up and letting go can be difficult for people, as described in a song by Joe Cocker. Provided they find the right host, viruses don't seem to have that problem despite many barriers such as plasma membrane, cytosol, or nuclear envelope. They have developed many courting strategies to enlist the assistance of host factors to deliver and express their secrets, namely the information coded in their genome. This is remarkable considering the fact that usually this courtship does not end well with the cell being deserted and devastated by a revitalized virus.

HIV enters cells expressing the appropriate receptors by fusion of its envelope with the plasma membrane (Fig. 1). During passage through the cytosol, the viral RNA genome is reverse transcribed into DNA in a structure named the reverse transcription complex (RTC). An unusual triple-helical DNA domain and the viral integrase, possibly in concert with matrix protein and Vpr, are responsible for importing the RTC into the nucleus, where the HIV genome is integrated into a chromosome (Whittaker et al., 2000; Greene and Peterlin, 2002). Tom Hope and his colleagues (McDonald et al., 2002) address an until now neglected aspect of HIV infection—the cytosolic events after fusion at the plasma membrane and before genome import into the nucleoplasm. It has been notoriously difficult to decipher the intimate relationship of incoming virions and host cell, since the signals to be interpreted are low, and the inoculum may contain defective particles that also show up by some detection methods.

McDonald et al. (2002) imaged intracellular HIV in living cells by incorporating a GFP–Vpr fusion protein into the virions. To ensure that they could distinguish functional cytosolic virus cores from nonfused virions, the viruses were labeled in two other ways. The HIV membrane was labeled by a lipophilic dye, DiD, that was incorporated into the envelope during virus assembly. Viruses that have undergone functional entry into the cytosol by fusion with a cellular membrane would be expected to lose their viral membrane. However, endocytosed virions may not be visible, since endocytic hydrophobic proteins could extract DiD from the membrane, or its fluorescence might change due to the low endocytic pH or hydrolysis. Moreover, the inoculum also contained some particles labeled with GFP but not by DiD. Thus, to identify functional cytosolic cores, McDonald et al. (2002) microinjected cells with fluorescent dUTP that is incorporated into the nuclear DNA as well as into the newly synthesized viral DNA present in the RTCs. Although there is the remote possibility that some dUTP leaked into the medium and was internalized, endocytic RTCs would have little access to the other nucleotides also required for reverse transcription. Thus, these elegant experiments achieved for the first time the characterization of functional, cytosolic RTCs.

In vivo fluorescence microscopy demonstrated that GFP–Vpr-labeled subviral HIV particles colocalize with MTs, move in curvilinear paths in the cytoplasm, and accumulate around the MT-organizing center. MTs are the cytoskeletal highways responsible for long distance transport of host as well as viral cargo, whereas actin filaments are implicated in short distance motility (Sodeik, 2000; Smith and Enquist, 2002). Previous experiments also confirmed in this study showed that HIV infection is reduced twofold if cells are infected in the presence of a MT-depolymerising drug nocodazole (Bukrinskaya et al., 1998). Viral gene expression of herpes simplex virus and adenovirus 2, whose capsids also utilize MTs for transport to the nucleus, is reduced about tenfold in the absence of a MT network (Mabit et al., 2002).

This discrepancy might reflect an alternative transport mechanism that is used most efficiently by HIV. McDonald

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*Abbreviations used in this paper: GFP, green fluorescent protein; HIV, human immunodeficiency virus; MT, microtubules; RTC, reverse transcription complex.

The Journal of Cell Biology, Volume 159, Number 3, November 11, 2002 393–395
http://www.jcb.org/cgi/doi/10.1083/jcb.200210024

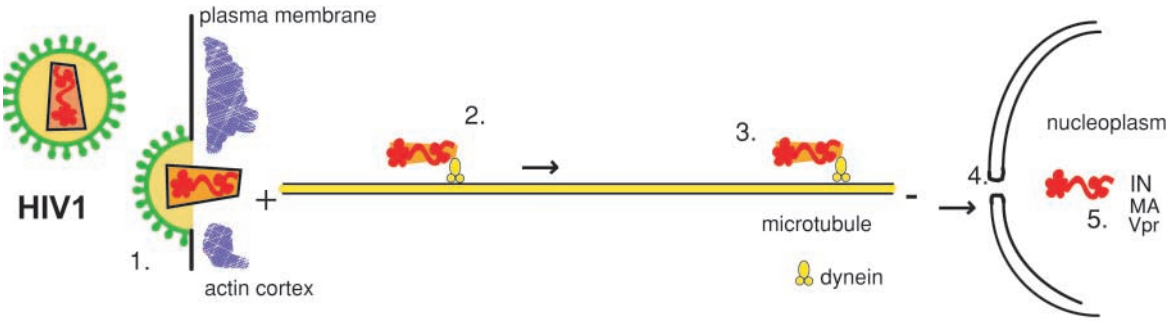

Figure 1. **Schematic description of the cell entry and uncoating of HIV.** HIV enters cells by fusion of its envelope (green) with the plasma membrane (1). The viral core (orange) and associated proteins are released into the cytosol. The viral RNA genome is reverse transcribed into DNA. The reverse transcription complex (red) is propelled along microtubules by dynein toward the microtubule minus-end–localized close to the cell nucleus (4). DNA and associated proteins are imported into the nucleus (5), where the viral genome is integrated into a host chromosome (modified from Sodeik, 2000; Whittaker et al., 2000).

et al. (2002) report that HIV transport was completely blocked in the presence of both nocodazole and latrunculin B, the later causing disassembly of the actin filaments. HIV motility resumed if either drug was washed out. However, the experiments were also performed differently: HIV gene expression was measured after two hours treatment followed by two days without nocodazole, whereas herpes simplex virus and adenovirus-infected cells were kept in the drug during the entire experiment. Thus, the challenge is still to demonstrate that MTs are required for efficient nuclear import or integration of the HIV genome.

Cytoplasmic dynein and kinesins, respectively, are the trucks or so-called motor proteins for transport either to the minus-ends of MTs at the MT-organizing center, or toward the plus-ends in the cell periphery (Sodeik, 2000). McDonald et al. (2002) demonstrated that the GFP-tagged HIV particles are transported predominantly inwards by measuring the distance of HIV particles from the plasma membrane and the nucleus over time. Moreover, when they microinjected a dynein function–blocking antibody, the relative transport toward the nucleus is significantly reduced. Instead, RTCs seem to accumulate in the cell periphery, suggesting a plus-end–directed MT transport. A candidate motor for this transport is KIF-4, a kinesin that can bind to the matrix protein of HIV and SIV (Tang et al., 1999). McDonald et al. (2002) report that experiments using a higher time resolution than in this work suggest that GFP-tagged HIV particles have peak velocities of 1 μm/s, consistent with rates measured for MT transport of cytosolic and viral cargo (Sodeik, 2000; Smith and Enquist, 2002).

Adenovirus and herpes simplex virus capsids also accumulate in the cell periphery if dynein transport is blocked by destroying dynactin, a cofactor required for many dynein mediated MT transport processes (Suomalainen et al., 2001; Döhner et al., 2002). One interesting question is whether HIV also requires the assistance of dynactin, or whether HIV RTC can bind directly to dynein as has been suggested for rhodopsin bearing vesicles (Tai et al., 1999). Adenovirus stimulates two distinct signaling pathways that promote minus-end–directed MT transport and enhance nuclear targeting, one activating protein kinase A and the other p38/MAP kinase (Suomalainen et al., 2001). It is tempting to speculate that p38/MAP kinase incorporated into virions could do a similar job for incoming HIV (Jacque et al., 1998).

There is a growing list of viruses, at least those requiring nuclear import for replication, that seem to use dynein to approach the MTOC (Whittaker et al., 2000; Smith and Enquist, 2002). One might therefore ask whether MT transport represents some kind of antiviral cellular response. Among the physiological cargo of dynein are particles of aggregated protein as large as 200 nm that are transported to the MT-organizing center where they are packed into aggresomes (Garcia-Mata et al., 1999). These aggresomes recruit chaperones for refolding but also proteasomes, cytosolic proteolytic machines that could clear such garbage from the cytosol. Moreover, aggresomes might be eliminated by autophagy that terminates in lysosomal degradation (Kopito, 2000).

Could it therefore be that, in the context of a viral infection, the cell treats a viral particle based on what it looks like: a large protein aggregate that should not be in the cytosol but sent for degradation? On the other hand, minus-end–directed MT transport seems to be the only possibility for a virus to get close to the nucleus. Viruses might therefore additionally enlist plus-end–directed MT motors to avoid the proteasomes and autophagosomes concentrated around the MT-organizing center, or at least to reduce the time spent in that hostile area. Interestingly, proteasome inhibitors increase the efficiency of HIV infection (Schwartz et al., 1998). The second potential function of plus-end–directed MT viral capsid transport could be to move from the MT-organizing center to the nearby nucleus.

McDonald et al. (2002) determined, for the first time, the ultrastructure of HIV RTCs during transit to the nucleus. For this, they used a technology perfected by Tatyana Svitkina and Gary Borisy that allows the alignment of fluorescent and electron microscopy images of individual detergent extracted cells. RTCs, identified by the incorporated fluorescent dUTP, were typically cylindrical in shape with varying length of 400 up to 700 nm and a diameter of ~100 nm. It will be interesting to determine whether native RTCs, derived from fusion at the plasma membrane, will have a similar morphology to those derived from the VSV-G protein pseudotyped HIV used by McDonald et al. (2002), which

fuse with an endosomal membrane. The RTCs were clearly bound to MTs, occasionally linked by stalk-like projections similar to the stalks described on herpes simplex virus capsids (Sodeik et al., 1997).

The RTCs detected by electron microscopy (McDonald et al., 2002) are considerably larger than the diameter of 56 nm measured for purified RTCs (Greene and Peterlin, 2002). This poses a serious problem as to how to access the nucleoplasm, since the size limit for nondeformable cargo that can pass through the nuclear pore is ~28 nm (Whittaker et al., 2000). Vpr present in the HIV virion can cause herniations in the nuclear envelope that upon transient rupture result in the mixing of nucleoplasm and cytosol. This suggested a provocative, although unproven, hypothesis for RTC nuclear entry, since the nuclei were shown to contain cytosolic markers after resealing (Noronha et al., 2001).

However, Vpr-negative HIV can also initiate infection, demonstrating that we still lack much in understanding HIV entry and genome uncoating. With the powerful approaches developed by McDonald et al. (2002) and the incredible progress in imaging single fluorescent molecules in living cells (Seisenberger et al., 2001), these important and fascinating questions of HIV cell biology can now be addressed.

Many thanks to Katinka Döhner, Thomas F. Schulz, and Rudi Bauerfeind for insightful comments on the manuscript.

Submitted: 7 October 2002
Accepted: 18 October 2002

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