Extended Abstract
Current research in Virology & Retrovirology
2020
Vol. 1, Iss. 1

12th World Congress on Virology and Infectious Disease, March 18-19, 2019, Amsterdam, Netherlands- Influenza A virus capsid disassembly: How a hard nut cracks itself during cellular entry

Indranil Banerjee
Indian Institute of Science Education and Research, India

Viruses are masters of camouflage and deception despite being very simple in structure and composition. Devoid of any means of independent locomotion, they disseminate by exploiting cells and organisms. A critical moment occurs when an epidemic particle reaches a possible host cell and attaches itself to the surface. It must now deliver its genome and accessory proteins into the host cell for replication. Unable to realize access to the cell on their own, viruses have evolved elegant strategies to extract assistance form the host cell for his or her entry and genome release. Influenza virus, one among the foremost devastating human pathogens, uses a singular trick to urge its capsid unpacked within the host cell. It mimics misfolded protein aggregates by carrying unanchored ubiquitin chains, and thereby activates the ubiquitin- and HDAC6-dependent protein degradation machinery (aggresome) of the cell. Upon activation, diverse components of the aggresome processing machinery including the microtubule- and actin-associated molecular motors dynein, dynactin and myosin 10, generate physical forces to crack open the viral capsid and release the viral RNA genome into the cytosol for replication. This cytoskeleton motor-assisted capsid disassembly program not only provides yet one more interesting insight on how viruses manipulate the host cells to propagate, but also offers potential targets like myosin 10, dynein and HDAC6 for new antiviral strategies to combat this deadly virus.

Introduction: HIV is an enveloped virus that carries its RNA genome and associated viral proteins within a protein shell called the capsid (Welker et al., 2000). Upon engagement of CD4 and the chemokine receptor CCR5 or CXCR4, the viral membrane fuses with the plasma membrane of the host, depositing the viral core (defined here as the capsid and its contents) in the cytoplasm (Blumenthal et al., 2012). In order to establish infection, the virus must reverse transcribe its single stranded RNA genome into double stranded DNA, traverse the cytoplasm and cross the nuclear membrane, after which it integrates into the host chromosome (Bukrinsky, 2004). We now know that the capsid plays a key role in these processes and is critical for successful infection. It not only acts as a shield to guard the viral genomic material from pattern recognition and degradation (Lahaye et al., 2013; Rasaiyaah et al., 2013), but is additionally thought to facilitate reverse transcription (Jacques et al., 2016), engage with the nuclear pore complex (Burdick et al., 2017; Dharan et al., 2016; Matreyek et al., 2013), and direct integration site targeting (Ocwieja et al., 2011; Schaller et al., 2011; Sowd et al., 2016). In order to achieve many of these proposed functions, the capsid must interact with host proteins and small metabolites, as well as disassemble to release the viral DNA at the appropriate place and time (Campbell and Hope, 2015). The nature of this 'uncoating' process and the way it's influenced by host co-factors remains a key unanswered question in HIV biology.

Here we describe an in vitro single-molecule fluorescence imaging assay that permits us to follow the uncoating kinetics of many individual HIV capsids

Note: This work is partly presented at 12th World Congress on Virology and Infectious Diseases, March 18-19, 2020, Amsterdam, Netherlands

during a single experiment. This type of single-molecule analysis has the advantage that it can resolve intermediates within the disassembly pathway that are otherwise averaged call at traditional ensemble assays. By observing the properties of many individual capsids, it is possible to classify them according to their uncoating behaviors. This versatile method enables 'bottom-up' approaches to determine the effect of individual host molecules and drugs and is also compatible with 'top-down' studies in which the influence of whole-cell lysates can be observed. Using this method, we are able to classify virions into three categories based on their uncoating behavior. We have also been able to define two discrete uncoating events that we term 'capsid opening' and 'lattice disassembly' and show that known cofactors and drugs have different effects on these two processes. This observation has allowed us to resolve the ambiguity as to what is meant by 'uncoating' and how it can be influenced by external factors.

Results: Fusion of the viral particle with the plasma membrane marks the point in time when the viral core is first exposed to the cytoplasm, but the effects of cellular proteins and small molecules on the stability of the capsid lattice are largely unknown. Here, we designed a fluorescence imaging assay to pinpoint the time of capsid opening at the single-particle level after exposing capsids to biochemically different environments in vitro. We produced viral particles containing GFP as a solution phase marker using a proviral construct with Gag-internal GFP (Aggarwal et al., 2012; Hübner et al., 2007). GFP is expressed as part of the Gag polyprotein and released by proteolysis during maturation, whereby a fraction of GFP molecules are compartmentalized within the viral capsid, with the remainder enclosed outside the viral capsid but within the viral membrane (Mamede et al., 2017; Yu et al., 2013). As a control we also used constructs containing a mutation in the late domain of Gag that leads to a block in the abscission of viral particles from the producer cell. The number of GFP-positive particles in the cell supernatant decreased by 99.7% when viral release was blocked, confirming that essentially all GFP-positive particles released in the absence of the block represent viral particles. The fluorescent viral particles were biotinylated, purified by gel filtration and captured via streptavidin onto the surface of a coverslip modified with an inert polymer layer that prevents non-specific adsorption of viral particles and proteins. Using microfluidics, we then delivered an answer containing the bacterial pore-forming protein perfringolysin O (PFO). PFO efficiently permeabilized the viral membrane by assembling into characteristic ring-shaped membrane pores with a mean pore diameter of about 35 nm, consistent with its activity on cholesterol-containing membranes (Dang et al., 2005). These pores are sufficiently large to permit the passage of proteins while the viral core is retained within the perforated viral membrane allowing the core to be observed over time while it can undergo disassembly.

Note: This work is partly presented at 12th World Congress on Virology and Infectious Diseases, March 18-19, 2020, Amsterdam, Netherlands