On their way out: Structural HIV proteins team up before escaping from infected cells

The human immunodeficiency virus (HIV) infects and eventually destroys the immune cells of infected patients. This causes the life-threatening medical condition called acquired immunodeficiency syndrome (AIDS). A cure for HIV/AIDS does not exist, and treatment is costly and lowers quality-of-life. Basic scientific research thus remains necessary, to increase our understanding on HIV, all the more because the virus alters itself inside infected patients to evade administered drugs.

In our research, we focus on the viral Gag protein. Viral Gag is a structural protein that forms the protective shell around the viral genome (in the form of RNA) and gives the virus its shape. Inside HIV infected cells, viral Gag proteins are known to assemble at the cell membrane and then bud off from the cell eventually forming new infectious viruses.

Viral Gag can be divided into different domains that allow the protein to interact with other biomolecules. Previous studies have shown that intermolecular Gag-Gag interactions occur through the capsid domain (CA), Gag-membrane interactions through the matrix (MA) domain, and Gag-RNA interactions are mediated by the nucleocapsid (NC) domain. It remained, however, unclear whether and to what extent Gag proteins already team up in the early stages of virus assembly, before interacting with cellular membranes.

By attaching a green fluorescent probe (a molecular light bulb) to the viral Gag protein, its density, mobility and cluster size can be accurately assessed in live cells using fluorescence microscopy methods and advanced analysis techniques.

We addressed this question by carrying out experiments in living human cells that mimicked conditions of an HIV-infected cell, including a special version of the Gag protein that was tagged with a fluorescent marker (a molecular light bulb). Using ultrasensitive fluorescence microscopy and advanced mathematical analyses (based on image correlation spectroscopy), we could monitor how fast Gag proteins move about inside cells and how densely they are packed, either moving in clusters or by themselves, and whether cluster size correlates with Gag density.

In particular, we were able to identify two distinct populations of Gag species in the cytosol; a faster- and a slower-moving Gag. Altering part of the Gag protein (the NC domain) to disrupt its interactions with RNA, increased the cellular Gag mobility of the faster-moving species, which we also showed to consist of a single Gag protein molecule. Other alterations that affected intermolecular Gag-Gag interactions or membrane binding did not further increase mobility. Gag mobility is thus influenced Gag-RNA interactions in the cytosol prior to membrane trafficking and independent of any membrane interactions. Surprisingly, when we abolished all possible interactions (RNA, membrane and intermolecular interactions) within Gag by combining several alterations, Gag still diffused slower than would have been expected for a pure species moving about by itself. This suggests that, apart from RNA which functions as a scaffold for Gag oligomerization, other cellular proteins are actually involved in Gag trafficking and will form stable or transient Gag interactions before Gag reaches the cell membrane.

The slower-moving Gag species, on the other hand, contained clusters of Gag, the size of which depended on the total cellular protein density, as would be expected for any process driven by intermolecular interactions, as proposed previously for the capsid domain of Gag. Thus, HIV-1 assembly is not purely a membrane process, but rather starts already inside the cellular pool by a defined clustering mechanism. This might well be an important step in viral replication and infectious virus formation.

Publication

Live-cell observation of cytosolic HIV-1 assembly onset reveals RNA-interacting Gag oligomers.
Hendrix J, Baumgärtel V, Schrimpf W, Ivanchenko S, Digman MA, Gratton E, Kräusslich HG, Müller B, Lamb DC
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