MICROSCOPY BASED MONITORING OF HIV-1 NUCLEOCAPSID PROTEIN DURING THE EARLY STEPS OF INFECTION

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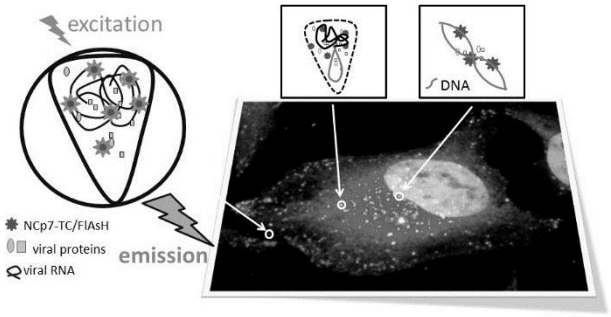

Figure 1:Fluorescence microscopy based monitoring of NCp7 release during the HIV-1infection

Fluorescence microscopy imaging of HIV-1 viruses requires a specific labeling of viral structures that minimally perturbs the infection process. Herein, we have produced an infectious HIV-1pseudovirus bearing NCp7 protein fused to a tetracystein tag that binds specifically a biarsenical fluorescein derivative (FlAsH). NCp7 is a nucleic acid chaperone that binds to the viral genome. After cellular entry, NCp7 assists reverse transcription. During this step, the viral complex undergoes important transformations to become the pre-integration complex (PIC) that is able to enter the nucleus and integrate the viral genome into the host cell DNA.

Single particle imaging of individual pseudoviruses with defined ratios of TC-tagged to non tagged NCp7 proteins, together with theoretical modeling of energy transfer between FlAsH dyes, showed that a high packaging of TC-tagged proteins in the viral cores causes a strong fluorescence quenching of FlAsH. Hence, by using confocal microscopy and image processing, we have been able to quantitatively monitor the concentration changes of NCp7 molecules in the viral complexes during infection. We have evidenced an important release of NCp7 molecules during the first 16 hours post infection. This release was dramatically decreased when reverse transcriptase was inhibited, showing its connection to viral DNA synthesis. A spatial analysis further showed that NCp7 release is more pronounced in the perinuclear space, where capsid disassembly is thought to be completed. Our data evidence the release of NCp7 during the viral transformation in the host cell cytoplasm.

In parallel, we established a protocol to image the viral pseudoparticles by Correlative Light-Electron Microscopy (CLEM) based on ReAsH induced DAB photoconversion. We visualized the viral cores in the cytoplasm of infected cells during the early steps of infection by TEM. This work describes for the first time a specific contrast given by HIV-1 viral proteins in TEM images and opens new perspectives for the use of CLEM to monitor the intracellular traffic of HIV-1.