Visualizing Interactions Between HIV-1 Nef and Host Cellular Proteins Using Ground-State Depletion Microscopy

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FIG. 1. Visualizing the Nef/MHC-I interaction along Rab5-positive endosomes. Here we imaged a HeLa cell utilizing ground-state depletion microscopy. Cells were cotransfected with plasmids expressing the HIV-1 accessory protein Nef and MHC-I fused to bimolecular fluorescence complementation (BiFc) fluorophores. Cells were subsequently stained for Rab5. The Nef/MHC-I BiFc signal (*green*) is distributed within the cell, colocalizing with Rab5-positive endosomal structures (*red*). The scale bar represents 2.5 μm .

THE HIV-1 ACCESSORY PROTEIN NEF is essential for HIV disease progression and pathogenesis. A major Nef function is the downregulation of cell surface MHC-I, which contributes to the ability of HIV-1 to evade immune surveillance by cytotoxic T cells.¹ This function is mediated by multiple Nef binding interfaces that interact with the cytosolic tail of MHC-I and multiple membrane trafficking regulator proteins, including the heterotetrameric adaptor protein 1 (AP-1) and phosphofurin acidic cluster sorting proteins 1 and 2 (PACS-1 and 2).¹ Both AP-1 and PACS-1 have been demonstrated to mediate the trafficking of cargo proteins between early endocytic vesicles and the trans-Golgi network. However, the specific cellular compartments implicated in Nef-mediated trafficking of MHC-I away from the cell surface are largely undercharacterized.

Our research program utilizes bimolecular fluorescence complementation (BiFc) to study the interaction between Nef and cellular binding partners. BiFc is a powerful tool for assessing the interaction of two protein-binding partners fused to nonfluorescent fragments of a split fluorescent protein, with interactions between the two proteins driving reconstitution of a functioning fluorophore.² The BiFc fluorophore reconstitution occurs only when both proteins of interest are within 10 angstroms. Therefore, this technique permits the visualization of protein–protein interactions and the identification of distinct subcellular compartments where the interaction occurs. Indeed, we have previously used BiFc to demonstrate that Nef interacts with PACS-1 and PACS-2 at both early and late endosomes, and that this interaction is critical for MHC-I downregulation.³

In Fig. 1 we have combined BiFc with ground-state depletion superresolution microscopy (GSDM) to study the interaction between Nef and MHC-I. GSDM images are acquired by repeatedly imaging a sample while stochastically switching fluorophores in and out of a nonfluorescent dark state. By mapping the position of the active fluorophores at high precision in each acquisition, images with a resolution of up to 20 nm can be formed at 10 to 20 times better resolution than confocal microscopy.⁴ When combined with our recently developed algorithms, we can begin to spatially segregate protein interactions within the cell. Figure 1 demonstrates the interaction between Nef and MHC-I via BiFc, with GSDM determining that this interaction occurs partially within Rab5-positive early endosomes. This result is consistent with previous findings that Nef reroutes MHC-I from the cell surface through the endocytic network.³ This image illustrates for the first time that Nef and MHC-I interact along early endosomes and contributes to our understanding of

the trafficking itinerary undertaken by MHC-I away from the cell surface.

In summary, by coupling the powerful techniques of BiFc and GSD microscopy, our research demonstrates a novel method to study HIV-1 Nef interactions at a per cell level. The analysis of such images will enable us to understand the location of specific protein complexes utilized by Nef to mediate MHC-I downregulation.

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Author Disclosure Statement

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