

MECHANISMS OF CD4 DOWNREGULATION BY THE Nef PROTEIN OF IMMUNODEFICIENCY VIRUSES

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The Nef protein encoded by the primate lentiviruses, HIV-1, HIV-2 and SIV, is critical for the progression from infection to AIDS. Nef is an accessory factor that is produced early after infection and regulates various signaling and trafficking pathways in the host cells, T-lymphocytes and macrophages/monocytes. The best-characterized function of Nef is the downregulation of CD4, an event that prevents superinfection and enhances virion release. Nef has been postulated to alter CD4 trafficking by linking the cytosolic tail of CD4 to three clathrin-associated, heterotetrameric adaptor protein (AP) complexes, AP-1, AP-2, and AP-3, which mediate protein sorting events at the *trans*-Golgi network (TGN), plasma membrane and endosomes, respectively. Using RNAi, however, we have found that Nef-induced CD4 downregulation is dependent on clathrin and AP-2, but not AP-1 or AP-3. This finding indicates that Nef downregulates CD4 mainly by inducing its accelerated endocytosis from the cell surface by a clathrin/AP-2-dependent mechanism. Using various protein interaction assays, we have found that Nef binds directly via dileucine and diacidic motifs to a hemicomplex composed of the alpha and sigma2 subunits of AP-2. In addition, we have used mutational analyses to identify the binding sites for the dileucine and diacidic motifs on the AP-2 alpha-sigma2 hemicomplex. Furthermore, we have demonstrated the cooperative assembly of a CD4-Nef-AP-2 complex as an intermediate in the mechanism of Nef-induced CD4 downregulation. Finally, we have shown that Nef has an additional function of targeting CD4 for ESCRT-dependent but ubiquitination-independent sorting to the multivesicular body pathway. These findings indicate that Nef acts as a multifunctional adaptor to link CD4 to the endocytic and lysosomal targeting machineries.