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Review

# Role of HIV-1 Nef protein for virus replication *in vitro*

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**Abstract**

The Nef protein of primate lentiviruses (simian and human immunodeficiency viruses; SIV/HIVs) appears to be multi-functional and plays a pivotal role in viral persistence and pathogenesis *in vivo*. Of its numerous functions reported to date, the ability to enhance virion infectivity in indicator cell lines and to augment viral replication in peripheral blood mononuclear cells (PBMCs) and lymphocytes (PBLs) is very well conserved among various SIV/HIVs. This review summarizes and organizes current knowledge of HIV-1 Nef with respect to this particularly virological activity for understanding the basis of its *in vivo* function.


*Keywords:* Nef; HIV-1; SIV; Replication *in vitro*

## 1. Introduction

The *nef* gene is present in primate lentiviruses but not in the other retroviruses. Early studies have demonstrated the importance of Nef for efficient viral replication and pathogenesis *in vivo*; *nef*-deleted SIVmac239 displays attenuated viral replication and pathogenicity in rhesus macaques [1]. Mutations and deletions of HIV-1 Nef also have been found in virus isolates from several long-term non-progressors of HIV-1 infection [2,3]. Even though it has been reported that *nef*-deleted HIV-1 [4] and SIVmac239 [5] finally induce AIDS symptoms, Nef is obviously required for maintenance of high viral loads in individuals, and accelerates the disease progression.

Nef appears to be a multi-functional protein that involves in: (1) down-regulation of cell surface molecules such as CD4, major histocompatibility complex class I and class II, CD28, and CD3, (2) enhancement of virion infectivity and stimulation of viral replication, and (3) modulation of T cell activation state (for review, see reference [6]). These functions are well conserved among primate lentiviruses, but the ability to down-regulate CD3 is lost in viruses of the HIV-1 lineage. It has been proposed that the inability of HIV-1 Nef to down-regulate CD3 leads to increased sensitivity of T cells for immune activation and to programmed cell death (for review, see references [6,7]). Functional contribution of each activity of HIV-1 Nef described above to HIV-1 pathogenesis *in vivo* remains to be determined.

Here, we focus on our current knowledge of Nef-mediated enhancement of virion infectivity in indicator cell lines (artificially generated target cells) and stimulation of viral replication in PBMCs and PBLs (natural target cells), which would affect virus spread and pathogenesis *in vivo*.

## 2. Characteristics of HIV-1 Nef

Nef is a membrane-associated phosphoprotein that is synthesized abundantly in early stage of viral infection. Nef is incorporated into virions and associates with viral core. Numbers of reports have shown that HIV-1 Nef interacts with various cellular proteins via distinct domains (Fig. 1). Of

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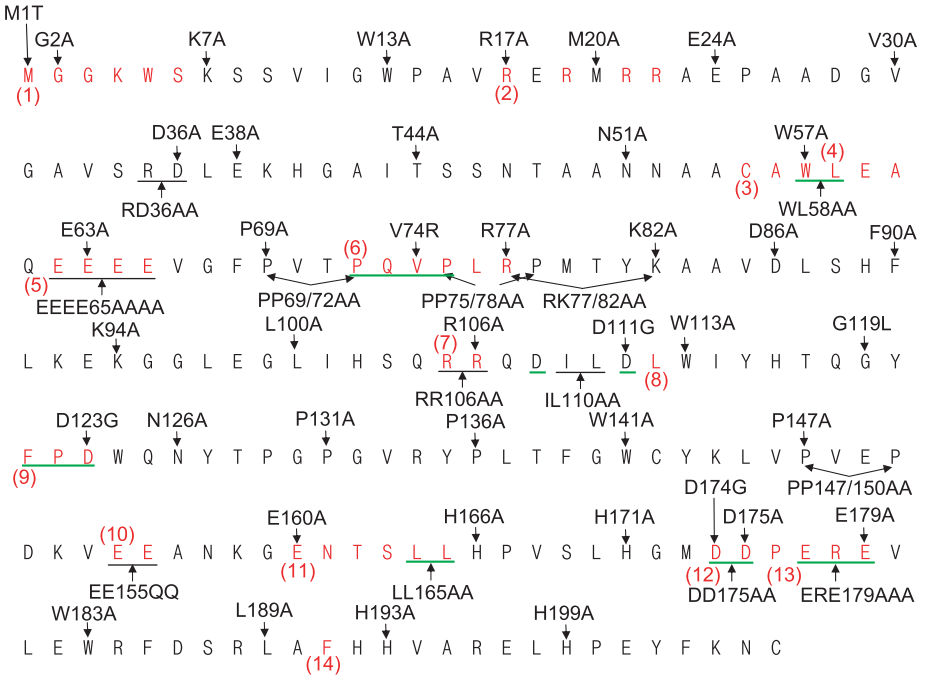


Fig. 1. Amino acids in HIV-1 Nef important for its functionality. Amino acid sequence of HIV-1 Nef from NL4-3 (GenBank accession no. AF324493) and the functionally important regions are shown. Position of site-specific mutations and designation of the mutants are also shown by arrows. Green underlines indicate the regions already reported to be involved in infectivity enhancement of virions and/or stimulation of viral replication in PBMCs and PBLs. Red colored amino acids and numbers represent the motifs of Nef that interact with or important for: (1) N-myristoyl transferase and Dynamin2, MGxxxS; (2) membrane targeting, R17/19/21/22; (3) HIV-1 protease, CAW↓LEA (cleavage site); (4) cytoplasmic tail of CD4, WL; (5) PACS-1 (Phosphofurin acidic cluster sorting protein-1), EEEE; (6) SH3 domains of Src family kinases, PxxPxR; (7) Pak1/2 (p21-activated kinase-1/2) and Nef multimerization, RR; (8) Pak2 and Nef multimerization, L; (9) human thioesterase and Dynamin2, FPD; (10) β-COP (β-coat protein), EE; (11) adaptor proteins AP-1/2/3, ExxxLL; (12) V1H (catalytic subunit H of vacuolar ATPase), DD; (13) c-Raf1 kinase, DDPxxE; (14) Pak2, F. X indicates any amino acids.

various functional roles assigned for Nef, the membrane-binding ability, which depends on its N-terminal myristoylation, is critical for all activities such as CD4 down-regulation and enhancement of virion infectivity. Mutational analysis of HIV-1 NL4-3 and SF2 *nef* alleles in the proviral construct has revealed that some regions are required for infectivity enhancement of virions in indicator cells and/or for stimulation of viral replication in PBMCs and PBLs [6,8–13]. These include $WL_{57,\ 58}$, $P_{72}xxP_{75}$, $D_{108}D_{111}$, $FPD_{121,\ 122,\ 123}$, $LL_{164,\ 165}$, $DD_{174,\ 175}$, and $ERE_{177,\ 178,\ 179}$ (Fig. 1). The domains involved in the other functions of HIV-1 Nef are summarized in references [6,11,13]. In order to give a general picture of the contribution of Nef to viral infectivity *in vitro*, we ourselves have generated a series of site-directed mutants throughout the *nef* gene in the context of HIV-1 NL4-3 proviral genome (Fig. 1). The resultant 55 mutants with single or multiple amino acid alterations were then monitored for viral infectivity in indicator cells. As shown in Fig. 2, overall, the mutational effects were rather small but distinct for some variants, confirming the previously published results. Even the mutants carrying multiple amino acid substitutions (such as EEEE65AAAA and ERE177AAA) showed a considerable infectivity. In addition, there appeared to be no large distinct regions or domains affecting much the viral infectivity *in vitro*. However, we have noticed that some point mutants (M1T, G2A, V30A, D111G, G119L, P136A, W183A, and L189A) show a low infectivity similar to that of the *nef*-minus virus (NL–Xh). These mutants were found to grow poorly in PBMCs in agreement with the results in indicator cells (Fig. 2), indicating the importance of the original wild-type amino acids in Nef for viral replication *in vitro*. Needless to say, the N-terminal myristoylation (M1T and G2A) and central multimerization (D111G) signals of Nef are critical for its activity (Figs. 1 and 2).

## 3. Enhancement of virion infectivity and stimulation of viral replication by HIV-1 Nef

As mentioned above, it has been suggested that Nef is an important factor for pathogenesis *in vivo* according to data from the macaque/*nef*-deleted SIVmac model and from analysis of human non-progressors infected with *nef*-deleted HIV-1. In contrast with the *in vivo* studies, initial works *in vitro* have labeled *nef* a negative regulatory factor, based on the observation that Nef down-regulates viral replication by suppressing transcription from HIV-1 LTR [14–16]. However, it has been well established now that Nef does promote modestly or affects little the viral spread in cultured cells. Nef shows positive effect on HIV-1 replication in PBLs particularly when the cells are infected with HIV-1 prior to cell activation [17,18].

In single-cycle replication assays, Nef modestly enhances virion infectivity [17,18]. Enhancement of the infectivity requires expression of Nef in virus-producer cells [19–21], suggesting that Nef may somehow modify HIV-1 virions