

# Nef: "Necessary and Enforcing Factor" in HIV Infection

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**Abstract:** The Human Immunodeficiency Virus -1 (HIV-1) Nef protein that was originally identified as a viral negative factor is a 27kDa myristoylated protein. However, this so called dispensable viral protein has emerged as one of the most important proteins for viral life cycle. Nef not only establishes the host cell environment suitable for viral replication and pathogenesis but also facilitates the progression of the infection into disease. Previous efforts have been focussed to explain how Nef down modulates host cell receptors like CD4 and MHC-1 molecules, thereby helping the virus to evade host defense and to increase viral infectivity. Nef also ably modulates specific processes like apoptosis in favour of viral life cycle other than being the stimulus for cell activation and signal transduction pathways. After much maligning over its reported positive or negative functions on the HIV-1 Long Terminal Repeat (LTR) promoter, the Nef protein is now perceived to enhance viral replication and infection through a combination of different effector functions. Recent reports emphasize a role for Nef in viral gene expression and place it in a prime position to oversee and optimize viral replication. Nef may do so by enhancing Tat mediated gene expression from the LTR by activating signalling pathways that result in a concomitant increase in the activation of general transcription factors, and also by mediating translocation of repression factors from the nucleus. Thus, Nef not only enhances infection but also plays an important role in viral replication and pathogenesis.

**Keywords:** HIV-1, Nef, LTR, gene expression, replication, pathogenesis.

The discovery of *nef* unlike other HIV genes was marked with enthusiasm so much so that the acronym stood for negative factor. Ahmad and Venkatesan [1] demonstrated that proviruses with mutations in their 3'ORF replicated better than the wild type virus in a transient expression system and that the mutant viruses maintained this enhanced replication status after several passages in T-lymphocytes. They further elucidated that the Nef protein in *trans* suppressed gene expression from the HIV-1 LTR, clearly a potential candidate for the control of HIV-1. This protein has since, been a subject of intense scrutiny with various studies describing functions as diverse as an inducer of signalling, to an inhibitor of apoptosis and enhancer of infection. These reports were supported by subsequent studies, which implied a negative role for the Nef protein in HIV-1 infection and replication [54, 57]. While a number of reviews have focused on the cellular signalling [31,78] and other well studied functions of Nef [5,24,30,102], a review updating the functions of Nef in transcription or gene expression from the HIV-1 LTR is not available. In this review, we have focused and brought into perspective the reported functions of Nef related to transcriptional activation and gene expression thereby implicating its role in HIV replication.

## GENERAL OVERVIEW

HIV-1, discovered as the etiological agent for AIDS, has a ~9.8 Kb genome encoding various proteins, of which none has probably intrigued the research fraternity more than the Nef protein [3]. The *nef* open reading frame present only in

primate lentiviruses, exhibits an important sequence polymorphism, and is actively transcribed early after infection. The earliest studies described it as a 27kDa membrane associated phospho-protein with a blocked myristoylated NH<sub>2</sub>-terminus [34]. Nef is one of the early proteins of the virus, which is expressed just after infection. It has been recently shown that expression of Nef initiates from pre-integrated viral genome [105] indicating again the importance of the protein in establishment of infection. As a protein, Nef has a few conserved amino acid stretches such as myristoylation signal, an internal methionine for translation initiation, a region of acidic charge, a PxxP repeat sequence, a potential protein kinase C phosphorylation site and arginine rich motif [4]. These motifs are not only conserved in different alleles of HIV-1 Nef but also in HIV-2 and SIV Nef, which are represented schematically in Fig. (1). These conserved regions have been shown to be quite important for this particular protein for the functions assigned to it. Following its identification, the *nef* gene was the center of unprecedented controversy regarding its function in the viral life cycle. Based on preliminary observations that a *nef* deleted virus replicated slightly better in T-cell lines as compared to the complete virus and also modestly repressed HIV transcription, Nef was proposed to "negatively" regulate the viral replication. This was followed by observations that replication of proviruses with overlapping deletions in *nef* was unaltered or enhanced over that of proviruses with intact *nef* [27]. More detailed studies followed, showing Nef as a transcriptional repressor of the HIV-1 LTR and mediating its effects *via* an upstream *cis* element recognized as the negative regulatory element (NRE) [17]. Nef expressing cells were reported to repress HIV-1 proviral expression and LTR transcription [57]. These reports were further supported by observations that Nef inhibits NF- $\kappa$ B induction and AP-1 recruitment in

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human T cells [65, 66]. On the contrary, certain other investigators claimed that Nef had no effects on viral replication and it did not exert negative effects on the HIV-1 LTR-directed transcription in human lymphoblastoid and myelomonocytic cells [36, 45].

Luria and colleagues noted that in a stably transfected T-cell line, Nef inhibited the accumulation of induced IL2 mRNA [56]. In a similar experimental system, Schwartz and colleagues noted no effect of Nef on CD4 expression and induction of IL2 production upon T-cell activation [87]. In contrast, the same investigators found that a ten fold higher expression of Nef by a retroviral vector reduced CD4 expression significantly [88]. Thus it appeared that a threshold level of Nef was required for its functions to be apparent. Alternatively, difficulties in maintaining Nef expression in stably transfected cells have been reported [44, 63], and prolonged cultures of T cells expressing a chimera of CD8-Nef led to the appearance of truncated forms of Nef [8]. Thus the concern was that upon stable transfection of *nef*, one might select for cells defective for Nef expression or for signalling deficient cells independent of Nef function. Subsequent reports indicated a positive effect of Nef in virus replication [45], a result, which was supported emphatically in a seminal study using functional *nef* mutants in a simian model of AIDS [43]. These studies were reinforced with data indicating more efficient replicative potential of clinically derived *nef*<sup>+</sup> viruses in activated peripheral blood mononuclear cells [21, 36, 45].

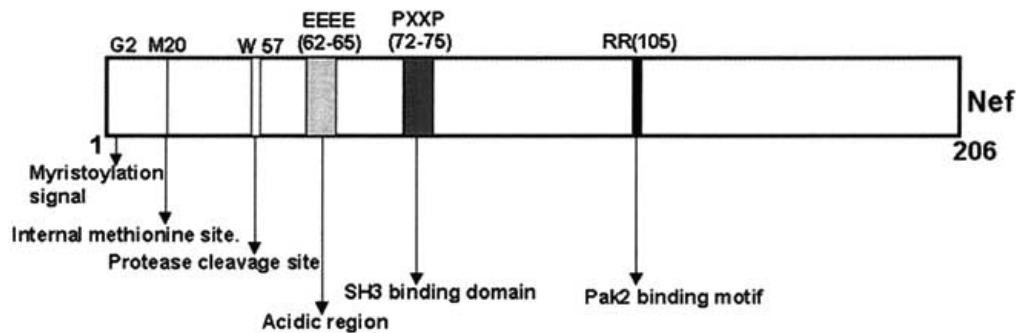
Viral changes such as deletions in the *nef* accessory gene have been correlated with long term non progression in HIV-infected individuals [22], however, three of the six members of the Sydney blood bank cohort harbouring *nef* deleted viruses have since progressed to AIDS [52]. In SIV, the Nef protein is required for induction of AIDS-like disease in monkeys [9] and the disease has slow progression in monkeys infected with *nef* deleted SIV [84]. This suggests that the presence of continued *nef* deletions cannot accentuate disease progression in HIV-infected humans and SIV-infected monkeys and that Nef is essential for efficient viral replication. This is supported by studies *in vivo* in severe combined immunodeficient mice engrafted with human immune systems (SCID-Hu mice) [40]. Micro array analysis has been employed to show that Nef is involved in signal transduction as well as in the transcriptional program [90, 91]. With regard to virally encoded transcriptional events,

the findings of Murphy *et al.* [63], and Ilyinski and Dresoriers [39] showing positive influence of Nef support an important role for Nef in viral gene expression. These studies also imply the complexities associated with the role of Nef protein in viral replication and thereby transcription from the HIV-1 LTR.

## ROLE OF NEF IN CELLULAR ACTIVATION

HIV replication in T cells is strongly influenced by T cell activation, which regulates crucial steps in virus life cycle. T cell activation supports viral life cycle by providing significant host cell factors which virus utilizes for its own replication. Opposing views persist till date despite extensive studies on the role of Nef in T cell activation [86,100]. The findings of Iafrate *et al.* [38] that Nef perturbs T cell receptor function is supported by some other observations where Nef down regulates major components of antigen induced T cell signalling like CD3, CD28 and CD4. However, convincing evidences implicate Nef in cellular activation and IL-2 induction [79]. Up regulation of IL-2 expression and subsequent autocrine stimulation by this cytokine are characteristics of T cell activation. Infection of a quiescent T cell line derived from macaques expressing either SIV or HIV Nef not only induces IL-2 production but also enhances virus replication 8-100 fold [2]. Nef mediated induction of IL-2 requires the conservation of myristoylation signal and SH3 – binding proline based motif [100]. Furthermore, culturing of macaque peripheral blood lymphocytes and a herpes virus saimiri-infected macaque T cell line require the addition of IL-2. Unlike lymphocytes, this cell line becomes IL-2 independent when infected by wild type SIV and not by *nef* deleted SIV [2]. This and many other findings provide support that Nef can play a direct, positive role in the T cell activation pathway [21, 61, 79, 93]. T lymphocytes from transgenic mice for Nef show hyper-responsive behaviour suggesting again that Nef can promote T cell activation [37,92].

The study of Nef mediated effects on T cell activation is not conclusive. There are several reports by which Nef is known to induce the cellular activation. Nef may regulate cellular activation through the cellular serine/threonine kinases as it has been shown to interact and activate p21 activated kinase (PAK) [67]. Nef expression dramatically increases nuclear factor of activated T cell (NFAT)



**Fig. (1).** Motifs in the Nef protein.

The PxxP motif is responsible for interaction with most signalling molecules. The myristoylation site is responsible for recruitment to the plasma membrane. The PAK binding domain mediates interaction with the PAK protein and subsequent rearrangement of the cytoskeleton.

transcription activity, which leads to up regulation of a number of activation-associated gene. In addition Nef also activates  $Ca^{2+}$ /calcineurin mediated signalling in T cells by a TCR independent mechanism. This activation of  $Ca^{2+}$  signalling is mediated by Nef and Ionositol triphosphate receptor (IP3R1) interaction, which further leads to the NFAT activation [59]. In primary CD4<sup>+</sup> T cells, the extracellular signal regulated kinase (ERK)- mitogen activated protein (MAP) kinase pathway is affected by Nef expression with an increase in ERK, MEK and Elk induction. This MAP kinase pathway activation is dependent on T cell receptor stimulation [85].

Nef, which lacks any catalytic activity, acts by influencing kinases or other signalling pathways within the host cell [94]. The strong conservation of proline rich motifs among various *nef* alleles and Src homology 3 (SH3) dependent activation of Src family tyrosine kinases may be a general property of primate lentiviruses. SH3 domains are modular protein units consisting of approximately 60 amino acids and serves to mediate protein-protein interactions involving the cellular signalling proteins such as the Src family of cytoplasmic protein kinases. In addition to Src, the family consists of Blk, Fyn, Fgr, Hck, Lck, Lyn, Yes and Yrk [78]. An important function of the Src family of kinases is to relay signals from the outside of a cell, which are mediated by transmembrane proteins lacking catalytic activity. Stimulation of Hck protein tyrosine kinase activity by HIV-1 Nef depicts a particularly extreme phenotype wherein a malignant transformation is observed in Rat2 fibroblasts [13]. This observation is relevant as Hck is rapidly induced following macrophage activation and has been implicated in multiple signalling events including phagocytosis, Fc receptor signal transduction, integrin signalling, and tumour necrosis factor release [23, 99].

Nef also disrupts normal TCR- initiated signalling by interfering with the CD3-TCR complex. HIV-1 Nef is also responsible for direct binding to CD28 and AP-2, which in turn induces endocytosis of CD28 *via* an AP-2 clathrin adaptor-dependent pathway [95]. This follows the report that chronic activation of HIV-1-infected CD4<sup>+</sup> T cells reduces expression of CD28 but increases expression of B7, thereby enabling these T cells to become antigen-presenting cells for uninfected CD4<sup>+</sup> T cells; this might be another mechanism for HIV-1 transmission *via* T-cell-T-cell contact [35].

Aggregation of lipid rafts initiates T cell signalling, similar to the manner of receptor stimulation by ligand. Nef might initiate the activation process by translocation to the plasma membrane, whereupon interaction with the lipid bilayer, a conformational change possibly occurs that permits the interaction with signalling proteins and primes for T cell activation [106]. Many of these signalling proteins, like Lck, or linker for activation of T cells (LAT), are at least partially present in glycolipid-enriched microdomains (lipid rafts) where Nef is also found. By binding to molecules of different compartments /rafts and possibly by forming oligomers, Nef may function as an intracellular cross-linker or adaptor. This is substantiated by the microarray analyses revealing about 97% of total expression profile in TCR activated T cell and Nef expressing cells are identical [91]. Based on this data, it has been proposed that Nef acts as a master switch early in viral life cycle making cellular environment favourable for virus production.

## INFLUENCE OF NEF ON HIV PATHOGENESIS

The *nef* gene of HIV is critical for AIDS pathogenesis and this is highlighted by the observation that some long term survivors of HIV are infected with strains containing deletions or defective alleles of the *nef* gene [49]. The role of Nef in AIDS pathogenesis has also been demonstrated by studies in animal models. SIV variants with *nef* deletions show reduced levels of pathogenicity [43]. In addition, transgenic mice expressing the Nef coding sequence of HIV-1 in CD4<sup>+</sup> T-cells develop a severe AIDS like disease which includes depletion of CD4<sup>+</sup> cells, weight loss, wasting, and premature death [37, 92]. The importance of Nef has also been demonstrated in the Rhesus-monkey model system of SIV-induced AIDS, where functional Nef expression is required for both high viral load and disease progression [43]. Of note is the difference observed in virus growth kinetics in primary as against immortalized T cells, which is probably due to the increased efficiency with which laboratory adapted strains of HIV propagate in immortalized T cell lines that might mask the difference in infectivity existing in each particle. This hypotheses gained credence as infection of the immortalized T cell lines at limiting dilutions of the virus does demonstrate greater infectivity for *nef*<sup>+</sup> HIV than for *nef* deleted HIV [61]. Using Hela-CD4-LTR- $\beta$ -galactosidase indicator cell line developed by Kimpton and Emerman [46], it was found that *nef*<sup>+</sup> HIV productively infected 5–20 fold more cells than an equal amount of *nef*<sup>-</sup> HIV [32,69]. This indicator cell line actually measures the production of Tat, expressed from the incoming virus genome after successful reverse transcription and integration, and transcriptional activation of LTR driven lacZ in the cell. Thus, the phenotype of increased infectivity shown by *nef*<sup>+</sup> HIV indicates an early role for Nef in Tat mediated transcriptional activation. Infectivity of *nef*<sup>-</sup> HIV can be rescued by expressing *nef* in *trans* in the virus-producing cell [60].

The best-known phenotypic effect of Nef *in vitro* is the down regulation of the cell surface expression of the CD4 receptor. The transmembrane glycoprotein CD4, which acts as the primary receptor for HIV-1 entry, is the target of down regulation by the viral protein Nef. Nef binds physically to the same dileucine motif in the cytoplasmic tail of CD4 to which the protein tyrosine kinase Lck (p56<sup>lck</sup>) binds, in the process targeting the CD4 receptor for endocytosis and transportation of CD4 to the lysosomal compartment. CD4 down regulation by Nef is thought to prevent premature death of infected cells due to superinfection [10,18]. It also seems to prevent interference in budding of progeny virus by inhibiting Env-CD4 interaction [82]. It has also been shown that CD4 down modulation may lead to enhancement of HIV-1 replication in activated T cells [55].

Adaptive immune responses against pathogenic viruses proceed *via* antigen presentation by the Major Histocompatibility Complex-I (MHC-I). To counteract this particular host defense mechanism, HIV-1 has developed strategies, which target the assembly and trafficking of newly synthesized MHC-I in infected cells. In the presence of Nef, MHC-I molecules traffic normally into the early Golgi apparatus. However, MHC-I molecules accumulate in the trans-Golgi network (TGN) and in post-Golgi clathrin containing vesicles of Nef expressing cells. MHC-I

downregulation is initiated by the binding of the Nef acidic cluster (EEEE 62-65) to PACS-1/AP-1. This is a crucial step to the subsequent PxxP 72-75 motif mediated ADP Ribosylation Factor 6 (ARF6) activation. Blockage of several steps in this activation process by phosphoinositol-3-kinase (PI3K) inhibitors indicates a role for PI3K in Nef mediated downregulation of MHC-1. Once MHC-1 is internalized into the Pq-sensitive ARF6 compartment, the alternative form of Nef facilitates sequestering of MHC-1 to the TGN. Thus, Nef *via* its different motifs hijack the ARF6 sorting pathway to rapidly remove the MHC-I-peptide complexes and sequester them in the TGN [11].

Apoptosis has been recognized for many years as a method used by the host immune system to defend against viruses. A major line of defense against virus infections is Cytotoxic T cells (CTL), which can recognize and induce apoptosis in virus-infected cells. Nef is one of the viral proteins, which could be responsible for evading this defense system of host. Nef induces FasL expression on infected cells, which leads to induction of cell death in bystander cells including CTLs. Simultaneously Nef inhibits Fas and TNF $\alpha$  induced apoptosis in infected cells by associating and inhibiting ASK1 [29]. In addition to this, Nef also blocks apoptosis in infected cells by inducing Bad phosphorylation in a PI3K and PAK - dependent pathway [104].

HIV also infects other cells of host immune system viz, macrophages and dendritic cells (DCs) using them as reservoirs for virus dissemination [15, 70, 71]. DC-SIGN is a dendritic cell specific lectin, an important surface molecule, which contributes in initiation of immune response by clustering DCs with T cells and is up regulated by Nef expression. Nef expression also promotes DC differentiation making them more competent APCs by up regulating surface molecules (CD1a, HLA-DR, CD40, CD83, CXCR4) as well as cytokines (IL1 $\beta$ , IL12, IL15, TNF- $\alpha$ ) and chemokines (MIP-1 $\alpha$ , MIP-1 $\beta$ ) [74]. Nef inhibits DC-SIGN endocytosis, leading to an increase in clustering of DCs with T lymphocytes thus disseminating virus to lymphocytes [51]. Macrophages have also been implicated in the spread of virus to CD4 $^{+}$  T cells. Swingler *et al.* [96] report that Nef expressing macrophages produce soluble ICAM-1 and soluble CD23, thus stimulating B cells to act as an intermediate in making resting, non cell-cycling T cells to become permissive for viral replication. In addition, infected macrophages were found to produce and secrete CC- chemokines (MIP-1 $\alpha$  and MIP-1 $\beta$ ), in a Nef-dependent manner. These chemokines attracted resting T cells and stimulated them for productive viral infection [97].

An interesting addition to the ongoing saga of emerging Nef functions has been its implication in remodelling actin to facilitate movement of the viral core past the cortical actin barrier [16]. Nef interacts and activates Vav, a cellular Rho GTPase exchange factor, responsible for cytoskeletal rearrangement. Sawai *et al.* first described two phosphoproteins with molecular weights of 62 kDa and 72 kDa observed in anti-Nef immunocomplexes after *in vitro* kinase reaction, and referred to these as Nef associated kinase activity (NAK) [83]. The identity of this kinase was initially revealed to be a p21- activated kinase (PAK) family member [67] and later as PAK2 [6, 77]. PAKs have direct effects on the cytoskeletal morphology, and PAK2 has been implicated

in apoptotic signalling. Nef may modulate the effects of PAK2 in mediating signals from the cytoplasm into the nucleus and/or in inducing reorganization of the actin cytoskeleton, both of which could facilitate steps such as transcription, reverse transcription or budding. It is also interesting to note that the form of PAK, which Nef binds i.e., PAK2, is the only member to be cleaved and activated by pro-apoptotic caspases. Although it has been demonstrated that Nef can associate with PAK2, involvement of other PAKs cannot be ruled out [6, 78]. Nef binding and activation of PAK related kinases and subsequent phosphorylation of its substrates can be detected in both primary T lymphocytes and macrophages. PAK-interacting guanine nucleotide exchange factor (PIX) is associated with its partner p95. PAK and PIX-p95 are differentially activated and phosphorylated depending on the intracellular environment in which Nef is expressed. As PIX-p95 is a novel effector in primary cells, it could suggest that the regulation of PAK signalling may be different in T cells and macrophages [14]. Class Ia phosphatidylinositol-3-kinases (PI3K) consist of a regulatory and a catalytic subunit. After the generation of Phosphatidylinositol-3,4,5-Pi, a variety of proteins are recruited to different membranes *via* their lipid binding domains. Among them are the exchange factors for small GTPases of the Rho, AFR family and the PDK-1 [108]. The relocalization of these proteins initiates a broad range of cellular effects like proliferation, membrane ruffling, prevention of apoptosis, and certain vesicular transport functions. Nef binds the regulatory p85 subunit of class Ia PI3K [104]. This interaction is functional as the selective inhibition of PI3K decreased the activity of PAK, which was co-precipitated by Nef. It might be so that Nef assembles PI3K, Vav, and PAK into a signalling complex, which is required for increased viral movement through the cortical actin in a Nef dependent manner [25, 73].

#### UPREGULATION OF TRANSCRIPTION FACTORS BY NEF EXPRESSION

HIV-1 gene transcription occurs from the LTR. The HIV-1 LTR possesses binding sites for numerous cellular transcription factors, including Sp1, NF $\kappa$ B, AP-1, and NFAT. Given that these factors are responsible for T cell activity, it is not surprising that T cell activation promotes viral gene expression. Stimulation of the TCR on mature T cells induces a series of biochemical events, which eventually result in the induction of gene transcription in the activated T cells. The initiating signals are the activation of the protein tyrosine kinases (PTKs), including members of the Src family, Syk/Zap-70 family and the Tec family. Recruitment and membrane localization of phospholipase C (PLC)- $\gamma$ 1 and PI3K permits phosphorylation of these proteins by PTKs and provides access to lipid substrates. Phosphorylation of PLC- $\gamma$ 1 results in the activation of its intrinsic enzymatic activity, subsequently leading to the hydrolysis of phosphatidylinositol-4,5-bisphosphate (PIP $_2$ ) to inositol-1,4,5-triphosphate (IP $_3$ ) and diacylglycerol. IP $_3$  generation induces a sustained increase in intracellular calcium while diacylglycerol promotes activation of protein kinase C (PKC). PKC may subsequently activate Ras by suppressing the activation of Ras-GTPase activator protein.

The elevation of intracellular calcium and stimulation of the Ras signalling pathway are sufficient for subsequent events required for T cell activation [101]. Thus the ultimate outcome of this complex series of signalling is the activation of transcription factors leading to increased gene expression. Besides promoting T cell activation, Nef mediated up-regulation of NFAT signalling could also contribute to HIV replication and AIDS pathogenesis by other mechanisms. Ectopic expression of NFATc in resting CD4+ T lymphocytes induced a permissive state, which despite the lack of evidence of T cell activation supported HIV replication in the absence of further stimulation [47]. IL-2 and FasL are the other important target genes whose induction relates to NFAT activation. NFAT has also been shown to activate HIV-1 LTR directed transcription by interacting with an unusual binding site that overlaps with the NF- $\kappa$ B- responsive element [48]. The observed effects by Nef on NFAT of course are in addition to the positive effects on the TCR, which is mediated by its interaction with an array of cellular kinases. The Nef mediated superinduction of IL-2 thereby is a reflection of activation of both NFAT and NF- $\kappa$ B sites and thus by this mechanism promoting viral replication and viral spread. Reiterating these effects is the study by Varin *et al.* describing the stimulation of HIV-1 LTR *via* NF- $\kappa$ B using exogenous Nef protein [98]. By using promonocytic U937 cells to study the effects of recombinant Nef protein, induction of various transcription factors AP-1, NF- $\kappa$ B and JNK were observed to lead to stimulation of viral replication in chronically infected promonocytic cells. Transcription factors reported to be up regulated due to Nef expression in T cells is presented in Table-1. Recent reports have also implicated the activation of STATs, i.e., STAT 1 and STAT 3 in human monocytes/macrophages *via* the release of soluble factors due to its interactions with the endocytic machinery [12,26].

## NEF INTERACTION WITH TAT LEADS TO ENHANCED VIRUS PRODUCTION

Immediately following infection, Tat is expressed at low levels, thus viral and cellular factors such as Nef or cytokines could amplify Tat function in enhancing viral replication during the early stages of HIV-infection. HIV-1 has the ability to replicate in quiescent PBMCs, and this ability is dependent on a functional *nef* gene. Nef may be essential for a permissive acute infection in the host, and may explain why the *nef* gene with premature stop codon of SIV<sub>mac239</sub> virus can revert to full-length open reading frame *in vivo*. This could be because of a strong requirement of the functional *nef* gene for the virus to establish an acute infection [72]. We have shown that *nef* enhances the gene-expression when expressed alongside Tat from the HIV-1 LTR. This is in addition to our observation that there exists a physical interaction between these two viral proteins and the enhancement of viral gene expression might be a result of this interaction [41]. Studies from our lab have also shown co-localization of these two proteins in the nucleus as also in the cytoplasm. These observations are in conformity with studies in B-cells stably transfected with Nef, which show presence of Nef in the nucleus [44]. A number of studies have further attempted to resolve the localization of Nef with a variety of cell types and conditions. It has been reported that even the myristoylated form of Nef is localized to the nucleus in addition to the cytoplasm thereby emphatically laying credence to the biological importance of the presence of Nef in the nucleus [50]. Studies by Murti *et al.* implicate the presence of a putative nuclear targeting sequence in the SF2 Nef sequence with the presence of Nef in distinct nuclear tracks [64]. These observations suggest implicitly that the Nef protein is transported along a specific pathway that extends from the nuclear envelope into the nucleoplasm. Immunohistochemical observations also

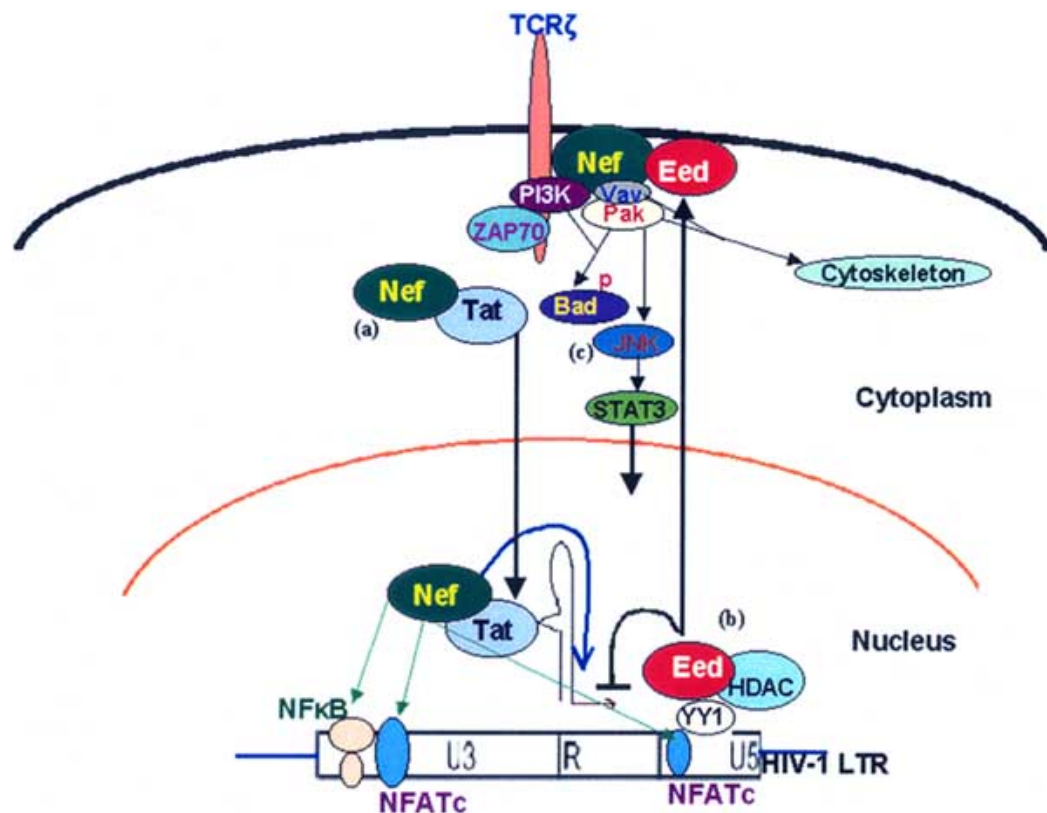
**Table 1. Enhanced Expression of Transcription Factors in T Cells Expressing Nef that Influence HIV 1 Gene-Expression**

Proteins	Functional significance	References
ATF 2	Increases HIV 1 transcription. DNA binding activity is increased in HIV-1infected T cell nuclear extracts.	[75]
CDK9	Up regulation of CDK9, a component of P-TEFb, which acts on Tat to facilitate progressive transcription.	[58, 107]
c-myb	Transcription factor expressed in proliferating T cells; specifically binds to HIV-1 LTR and influences viral gene expression.	[20]
c-fos	May bind to c-fos response element downstream of TAR in HIV-1 LTR and activate viral gene expression.	[80]
ETS 1	Interacts with NF- $\kappa$ B/NFAT proteins and mediate synergistic activation of HIV-1 and HIV-2 enhancers.	[7]
GABP $\alpha$	A purine-rich-repeat binding protein that enhances HIV-1 LTR mediated transcription and viral replication through Raf 1 Kinase.	[28]
IRF 1	Interferon regulatory factor 1 activates HIV-1 LTR both in absence or presence of Tat.	[89]
Pur- $\alpha$	A single stranded DNA binding protein binds to HIV-1 TAR RNA and activates HIV 1 transcription.	[19]
NFATc	Binds to U3 and U5 region of HIV-1 LTR and transactivates LTR mediated transcription through a binding site downstream of TAR region.	[81]
NF- $\kappa$ B p52	Binds to HIV-1 initiator region as homodimer to regulate certain preinitiation complexes.	[62]
TFIID	Enhances HIV-1 gene expression both by interacting with Tat and through NF- $\kappa$ B and Sp1.	[33, 42]
Tat SF1	A cellular elongation factor which serves as co- activator of HIV-1 Tat.	[53]

demonstrate the localisation of Nef in the nucleus of HIV-1 infected MT-4 and H-9 lymphoid cells [68]. Of particular interest is the study, which describes the presence of Nef in the cytoplasm in the regulatory phase (6-9hrs) and the subsequent presence of Nef and Tat in the nucleus in the productive phase (12-48 hours) post infection [76]. In light of these observations and with known transducing property of Tat, it is tempting to speculate that the interaction of Nef with Tat would enable the transfer of Nef to nucleus in proportions greater than would be possible as endowed by its inherent properties. Additional recent data supporting this observation have implicated Nef in recruiting the embryonic ectodermal development (Eed) protein, a negative regulator of gene expression from the nucleus to the cytoplasm [103]. The effect of Nef on the status of transcription can further be appreciated by the observation that the nuclear Eed protein associates with the HIV-1 LTR. Eed represses transcription *via* its association with HDAC 1 and 2 and the transcription factor YY1. YY1 was also found to bind and repress the HIV LTR *via* its association with the HDAC proteins. The translocation of the Eed protein from the nucleus thus coordinates signalling events at the membrane alongside alleviation of the transcriptional repression [103]. These events thus enhance the Tat mediated HIV-1 transcription from the HIV-1 LTR and also contribute to a general increase in transcription factors (Fig. (2)). These observations add another dimension to the emerging Nef saga in the process of transcription and gene expression.

### The Necessary and Enforcing Factor!

The sequence of events in establishing a productive infection by HIV follows mechanisms by which the virus takes over the cellular machinery. The relatively small Nef protein finds itself involved in the thick of things in the infection process. The association begins by its presence in the virion, and its importance in the entry process. Evidence from various studies now implicates the necessity and functionality of Nef in various steps of the infection and replication status of HIV-1. A current model as gleaned from the various studies on Nef indicates that the presence of Nef protein in virions is important for the entry of Nef into a susceptible cell. This function of Nef is implicated by the ability of Nef to rearrange the cortical actin network and also *via* its ability to interact with various cytoskeletal factors. Nef also interacts with signalling molecules on the membrane, thereby either differentiating the cells (immature dendritic cells) or activating cells (T cells) to make it receptive to viral infection. Nef then undertakes upon itself to play a character role in this ongoing saga by tampering with the cellular signalling machinery. It is endowed on the PxxP motif, which by its ability to bind to SH3 domains, virtually present in most signalling molecules proceeds to manipulate them to the dictates of the infection. This results in a variety of effects, the increase in down regulation of prominent cellular receptors, the up regulation of transcription factors, the modulation of anti- and pro-apoptotic signals. Finally the coup-de-grace is rendered by



**Fig. (2).** Model for Nef mediated up regulation of transcription factors.

a) Nef physically associates with Tat and enhances Tat mediated transactivation.

b) Nef also recruits the negative transcriptional regulator Eed to the cytoplasm thus relieving the inhibition leading to an increase in transcription.

c) Nef also activates various transcription factors *via* its interaction with host signalling molecules.

Nef acts at various levels to hijack the transcriptional machinery and swings the balance of infection to a productive state *via* an increase in HIV replication status.

the increase in viral production. This is enabled *via* a number of important events, activation of upstream signalling molecules, inhibition of apoptosis in the infected cell, transport of Nef to the nucleus, activation and up regulation of transcription factors, alleviation of repressors of transcription, enhancement of Tat mediated transcription *via* its direct association with Tat as also with the increase of the infectivity of newly produced virions. We therefore, propose a change in the acronym for "Nef" to Necessary and Enforcing factor as this truly depicts the complexity and intricate balance that this humble protein of 27 kDa brings to establishment and propagation of HIV-1 infection.

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